

# Contribution of Type II phospholipase A<sub>2</sub> to *in vitro* phospholipase A<sub>2</sub> enzymatic activity in human term placenta

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## Abstract

Although phospholipase A<sub>2</sub> (PLA<sub>2</sub>) enzymatic activities have been implicated in the regulation of phospholipid metabolism and eicosanoid formation in human gestational tissues, the role and contribution made by individual PLA<sub>2</sub> isozymes has not been established. The aim of this study, therefore, was to determine the contribution made by Type II PLA<sub>2</sub> to PLA<sub>2</sub> enzymatic activity present in human term placenta. The experimental paradigm used to establish the contribution made by Type II PLA<sub>2</sub> to total *in vitro* PLA<sub>2</sub> enzymatic activity present in placental extracts was to remove Type II PLA<sub>2</sub> by immunoaffinity extraction and then to quantify residual PLA<sub>2</sub> enzymatic activity. Before immunoaffinity extraction, Type II PLA<sub>2</sub> immunoactivity and total PLA<sub>2</sub> enzymatic activity present in placental extracts averaged 28.0 ± 10.0 ng/mg protein and 1040 ± 367 pmol/h per mg protein (*n*=3) respectively. After solid-phase immunoaffinity batch extraction of

placental extracts, immunoreactive Type II PLA<sub>2</sub> was not detectable by ELISA, and PLA<sub>2</sub> enzymatic activity was decreased by 82 ± 1% (*P*<0.001). Residual (i.e. non-Type II) PLA<sub>2</sub> enzymatic activity was further characterised by Western blot analysis and enzyme activity assay. The data obtained are consistent with a contribution by both cytosolic PLA<sub>2</sub> and other secretory PLA<sub>2</sub> isozymes (i.e. non-Type II) to residual PLA<sub>2</sub> enzymatic activity. The results obtained in this study support the conclusion that Type II PLA<sub>2</sub> is quantitatively the primary PLA<sub>2</sub> isozyme that contributes to *in vitro* PLA<sub>2</sub> enzymatic activity present in extracts of human term placenta, accounting for at least 80% of total activity. These data further support the involvement of this extracellularly active isozyme in the regulation of placental phospholipid metabolism and eicosanoid formation during late gestation.

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## Introduction

Phospholipases A<sub>2</sub> (PLA<sub>2</sub>) represent a family of esterases that hydrolyse the *sn*-2-acyl ester bond of 1,2-diacyl-*sn*-3-phosphoglycerides, liberating equimolar amounts of 1-acyl-glycerophosphatide and free fatty acid (Slotboom *et al.* 1978, Dennis *et al.* 1991). Obstetric interest in this signalling pathway has principally focused upon the role of phospholipases in liberating the fatty acid substrate, arachidonic acid, for the synthesis of eicosanoids, and in particular, prostaglandins. These phospholipid metabolites have been implicated in many aspects of pregnancy, fetal development and parturition (Thorburn & Rice 1988, 1990, Olson *et al.* 1992, López Bernal *et al.* 1993). In recent years, isozymes of PLA<sub>2</sub> that act intracellularly (cytosolic PLA<sub>2</sub>; cPLA<sub>2</sub>) or extracellularly (secretory PLA<sub>2</sub>; Type II, Type IV and Type V) have been identified in human gestational tissues (Aitken *et al.* 1990, Bennett *et al.* 1993, Andersen *et al.* 1994, Chen *et al.* 1994, Buhl *et al.* 1995, Freed *et al.* 1997). While the functional role of cPLA<sub>2</sub> isozymes in generating glycerophospholipid-

derived second messengers is consistent with our understanding of the action of these enzymes, the role of secretory PLA<sub>2</sub> isozymes that are released by human gestational tissues has yet to be clearly established (Rice 1995).

Although the expression of three secretory PLA<sub>2</sub> isozymes has been identified in human term gestational tissues, only one of these, Type II PLA<sub>2</sub>, has been studied in any detail. The data obtained to date establish the presence of Type II PLA<sub>2</sub> mRNA (Aitken *et al.* 1992, Bennett *et al.* 1993, Freed *et al.* 1997) and immunoreactive protein (Aitken *et al.* 1993) in placenta, choriondecidua and amnion. Consistent with its extracellular site of action, Type II PLA<sub>2</sub> is secreted by human placenta, *in vitro* (Farrugia *et al.* 1997) and is present in maternal plasma during pregnancy (Pulkkinen *et al.* 1993). Consistent with the possible involvement of this isozyme in the onset and/or progression of labour and delivery, the concentration of immunoreactive (ir) Type II PLA<sub>2</sub> in maternal plasma increases in association with labour, both at term and preterm (Rice *et al.* 1992).

Recently, the tissue-specific expression of Type II, Type IV and cPLA<sub>2</sub> mRNA transcripts was characterised in human term gestational tissues (Freed *et al.* 1997). In this study, Type II PLA<sub>2</sub> mRNA was reported to be the most abundant transcript present in human term placenta. These data support the hypothesis that Type II PLA<sub>2</sub> represents the major isozyme contributing to total PLA<sub>2</sub> enzymatic activity in this tissue. Previous studies have identified PLA<sub>2</sub> enzymatic activity in human term gestational tissues, but have not characterised the contribution made by the different PLA<sub>2</sub> isozymes present (Schultz *et al.* 1975, Grieves & Liggins 1976, Schwarz *et al.* 1976, Okazaki *et al.* 1978, López Bernal *et al.* 1992). Thus the aim of this study was to determine the contribution made by Type II PLA<sub>2</sub> to total PLA<sub>2</sub> enzymatic activity in human term placenta. Immunoaffinity batch extraction was utilised to remove Type II PLA<sub>2</sub> from placental extracts, and residual PLA<sub>2</sub> enzymatic activity was quantified and characterised.

## Materials and Methods

### *Tissue collection and processing*

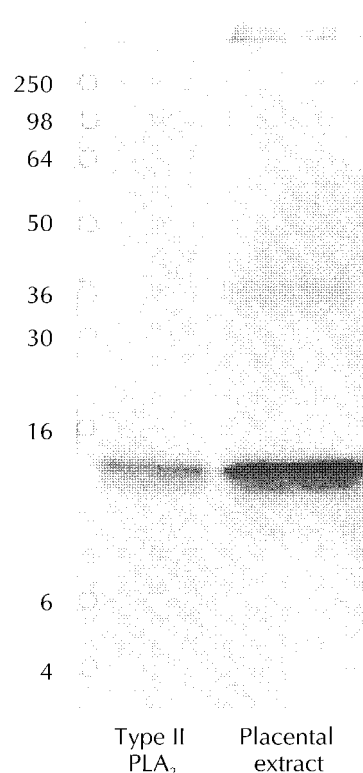
Human placentae ( $n=3$ ) were collected from women with uncomplicated singleton term (37–41 weeks of gestation) pregnancies at the Royal Women's Hospital (Melbourne, Australia) in compliance with and approval of the research and ethics committees of the hospital. Placental tissues were collected at elective Caesarean section (i.e. before the onset of labour) and were transferred to the laboratory within 10 min of delivery. Attached fetal membranes were removed and discarded. Placental tissue was frozen in liquid nitrogen and stored at  $-40^{\circ}\text{C}$ .

### *Tissue extraction*

Frozen tissues (10 g wet weight) were thawed and homogenised in 5 volumes of 1 M NaCl by  $3 \times 20$  s bursts with a metal blade homogeniser (Jenke and Kunkel, Staufen, Germany). The homogenate was centrifuged at 900 g (model J2-MC centrifuge; Beckman, Palo Alto, CA, USA) for 10 min. The supernatant was collected and dialysed using 6–8 kDa cut-off dialysis tubing (Spectra/Por; Spectrum Medical Industries Inc, Los Angeles, CA, USA) for 24 h at  $4^{\circ}\text{C}$  against 4.5 litres 0.01 M Tris buffer (pH 8.0) containing 150 mM NaCl. The retentate was assayed for PLA<sub>2</sub> enzymatic activity, PLA<sub>2</sub> immunoactivity and protein content (see below).

### *Immunoaffinity extraction of Type II PLA<sub>2</sub>*

Placental extracts were subjected to two cycles (3 h at  $4^{\circ}\text{C}$  and then 18 h at  $4^{\circ}\text{C}$ ) of solid-phase immunoaffinity batch extraction using mouse monoclonal antibodies raised



**Figure 1** Western blot analysis of human recombinant Type II PLA<sub>2</sub> standard (50 ng) and placental extract (20 µg protein) fractionated by SDS-PAGE under non-reducing conditions and probed with monoclonal antibody 4A1.

against (1) human recombinant Type II PLA<sub>2</sub> (4A1; Green *et al.* 1991) or (2) moth surface antigen (MSA, control extraction) coupled to agarose beads (Sephacrose 4B; Pharmacia, Uppsala, Sweden) using cyanogen bromide. After extraction, agarose beads were removed by centrifugation (900 g for 10 min) and the supernatant assayed for PLA<sub>2</sub> enzymatic activity, PLA<sub>2</sub> immunoactivity and protein content (see below). Data are expressed as percentage of pretreatment activity. The specificity of the monoclonal antibody was confirmed by Western blot analysis (Fig. 1).

### *Characterisation of residual PLA<sub>2</sub> enzyme activity*

PLA<sub>2</sub> enzymatic activity remaining after two cycles of immunoaffinity batch extraction (residual PLA<sub>2</sub> enzymatic activity) was characterised by establishing the effects of known inhibitors of secretory PLA<sub>2</sub> enzymatic activity: dithiothreitol (DTT; 12.5 mM) or EGTA (12.5 mM) (Clark *et al.* 1990). Data are expressed as percentage of control activity.

### *PLA<sub>2</sub> ELISA*

A non-competitive sandwich ELISA was used to measure PLA<sub>2</sub> content in extracted gestational tissue (Smith *et al.*

1992). Microtitre plates (96-well Maxisorp; Nunc, Roskilde, Denmark) were coated for 16 h at 4 °C with 100 µl purified monoclonal antibody raised against human recombinant Type II PLA<sub>2</sub> (9C1) at a dilution of 1:1000 in PBS. The wells were then blocked with 200 µl 1.0% (w/v) skimmed milk powder/0.1% (w/v) BSA in PBS for 3 h at 37 °C. The plates were then washed twice with 0.1% BSA in PBS. Aliquots (100 µl) of standard or diluted sample were added and incubated for 2 h at 37 °C. The plates were then washed. Purified monoclonal antibody 4A1 conjugated to alkaline phosphatase using glutaraldehyde was diluted 1:1000 in 0.1% BSA in PBS. Then 100 µl conjugated antibody was added to each well and incubated for 30 min at 37 °C. Plates were washed three times, followed by a further two washes with 20 mM carbonate buffer, pH 9.8. A 100 µl volume of the substrate *p*-nitrophenyl phosphate (1 mg/ml in carbonate buffer) was added to each well. The plates were placed at room temperature for at least 20 min and the absorbance at 405 nm was determined using a microplate reader (model 3350; Bio-Rad Laboratories, Richmond, CA, USA). A standard curve was generated by diluting purified recombinant PLA<sub>2</sub> in 0.1% BSA in PBS. Intra- and inter-assay coefficients of variation averaged 8.2 and 8.8% respectively.

#### PLA<sub>2</sub> enzyme assay

PLA<sub>2</sub> enzymatic activity was determined by the method of Green *et al.* (1991) as modified by Farrugia *et al.* (1993). Radiolabelled phospholipid 1- $\alpha$ -1-palmitoyl-2-[<sup>14</sup>C] arachidonylphosphatidylethanolamine (NEN Research Products, Dupont Australia, Sydney, NSW, Australia) was evaporated to dryness under nitrogen, resuspended in deoxycholate (in deionised water) and then diluted in Tris buffer (100 mM Tris, pH 8.0) containing 10 mM CaCl<sub>2</sub> and 300 mM NaCl before assay. Both substrate and samples were prewarmed to 37 °C before assay. Incubations were commenced by the addition of 25 µl phospholipid (0.22 nmol) to 25 µl diluted sample. Tubes were centrifuged briefly and then incubated for 1 h at 37 °C. Incubation mixture (30 µl) was then applied to a thin-layer chromatography plate (TLC) (Silica G; ICN, Costa Mesa, CA, USA). TLC plates were developed to a distance of 6 cm in chloroform/methanol/acetic acid (9:1:0.1, by volume). Radioactivity was localised by autoradiography (X-OMAT AR film; Kodak, Rochester, NY, USA), excised from the plates and quantified by liquid-scintillation spectrometry. Data are expressed as pmol phospholipid hydrolysed/h per mg protein (unless otherwise indicated). Intra- and inter-assay coefficients of variation averaged 9.8 and 14.3% respectively.

#### SDS-PAGE and Western blot analysis

The recovery of protein and irType II PLA<sub>2</sub> through tissue processing and extraction procedures was characterised by

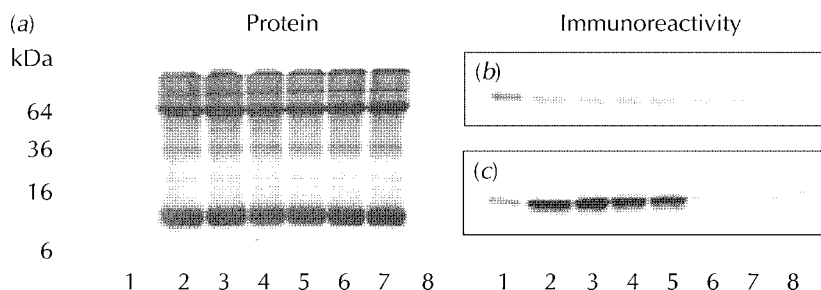
SDS-PAGE and Coomassie Brilliant Blue staining and by Western blot analysis. To negate the possibility of non-selective loss of PLA<sub>2</sub> during the procedure, the recovery of cPLA<sub>2</sub> was also monitored by Western blot analysis. Samples (20 µg protein) obtained at each stage of tissue processing and immunoaffinity extraction were diluted in loading buffer (125 mM Tris-HCl, pH 6.8; 4% SDS; 0.02% bromophenol blue; 20% glycerol), boiled for 5 min and then size fractionated on a 15% acrylamide gel under non-reducing conditions. Non-reducing conditions were used as the monoclonal antibody 4A1 recognises an epitope of Type II PLA<sub>2</sub> that is lost on reduction (data not shown). Molecular mass markers (Gibco-BRL, Grand Island, NY, USA) were run in parallel on all gels. Proteins were visualised by Coomassie Brilliant Blue staining or electrophoretically transferred to nitrocellulose membrane (NitroBind; Micron Separation Inc, Westboro, MA, USA), using a Hoefer semi-dry electroblotter (TE77, SemiPhor Transfer Unit) at 0.8 mA/cm<sup>2</sup> for 1.5 h. Nitrocellulose membranes were blocked with skimmed milk powder (0.5%, w/v) in Tris-buffered saline containing Tween 20 (0.05%, v/v; Sigma Chemical Co, St Louis, MO, USA) (TTBS) for 1 h at room temperature. Membranes were washed 3 × 10 min in TTBS and incubated with anti-Type II PLA<sub>2</sub> monoclonal antibody (4A1; 1:1000, v/v) or anti-cPLA<sub>2</sub> antibody (1:500, v/v; Genetics Institute, Cambridge, MA, USA) for 18 h at 4 °C. Membranes were washed (3 × 10 min) in TTBS and incubated with anti-mouse IgG-horse radish peroxidase conjugate (Silenus, Melbourne, Australia; for Type II PLA<sub>2</sub> development) or anti-rabbit IgG-horse radish peroxidase conjugate (Silenus; for cPLA<sub>2</sub> development) for 1.5 h at room temperature and then washed (3 × 10 min) in TTBS. Immunoreactivity was visualised using an enhanced chemiluminescence (ECL) procedure (Amersham, Arlington Heights, IL, USA).

#### Protein assay

The protein content of gestational tissues, homogenates and supernatants was determined by a protein dye-binding method (Bio-Rad Laboratories), utilising BSA as a reference standard (Bradford 1976).

#### Statistical analyses

Statistical computations were performed using a commercially available statistical analysis package (Statgraphics, STSC, Rockville, MD, USA). The homogeneity of data was tested using Bartlett's test (Bartlett 1937). Subsequently, two sample comparisons were analysed using Student's *t*-test, and three or more data sets were analysed using ANOVA. Statistical differences between individual group means were assessed using Student-Newman-Keuls tests. Statistical significance was indicated by a *P* value of less than 0.05. Data are expressed as mean ± s.e.m.



**Figure 2** SDS-PAGE of human recombinant Type II PLA<sub>2</sub> and placental extract (20 µg protein/lane). (a, c) Lane 1, 50 ng recombinant human Type II PLA<sub>2</sub>; lane 2, placental extract before dialysis; lane 3, placental extract after dialysis; lane 4, placental extract after first cycle of anti-MSA antibody treatment; lane 5, after second cycle of anti-MSA antibody treatment; lane 6, after first cycle of anti-Type II PLA<sub>2</sub> antibody treatment; lane 7, after second cycle of anti-Type II PLA<sub>2</sub> antibody treatment; lane 8, 25 ng PLA<sub>2</sub>. (a) Protein size fractionation and recovery before transfer to nitrocellulose and development using Coomassie Brilliant Blue. (c) Western blot analysis of irType II PLA<sub>2</sub> developed using ECL. (b) Western blot analysis of immunoreactive cPLA<sub>2</sub> developed using ECL. Loading is as described above with the exception that lane 1 contains rabbit lung cPLA<sub>2</sub> standard and lane 8 is empty.

## Results

### Placental tissue PLA<sub>2</sub> content

Before Type II PLA<sub>2</sub> immunoaffinity extraction, irType II PLA<sub>2</sub> and total *in vitro* PLA<sub>2</sub> enzymatic activity present in term placental tissue ( $n=3$ ) averaged  $28.0 \pm 10.0$  ng/mg protein and  $1040 \pm 376$  pmol/h per mg protein respectively.

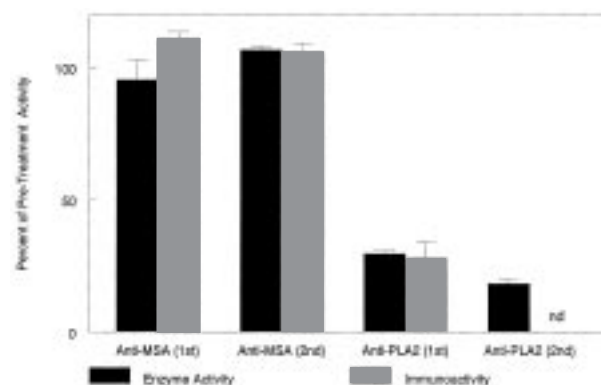
### SDS-PAGE and Western blot analysis

To determine the contribution made by Type II PLA<sub>2</sub> to total PLA<sub>2</sub> enzymatic activity, Type II PLA<sub>2</sub> was removed from placental extracts by solid-phase immunoaffinity batch extraction, and residual enzyme activity quantified and characterised. Initially, the effects of tissue processing and batch extraction on Type II PLA<sub>2</sub> and cPLA<sub>2</sub> immunoactivity and total protein were characterised by SDS-PAGE with Coomassie Brilliant Blue staining, and Western blot analysis respectively. A representative processing and extraction profile for proteins, PLA<sub>2</sub> irType II and cPLA<sub>2</sub> for samples of placental homogenate is presented in Fig. 2. No significant effect of tissue processing or immunoaffinity batch extraction on placental extract total protein content was identified (Fig. 2a). Using Western blot analysis, a Type II PLA<sub>2</sub> immunoreactive band, which co-migrated with human recombinant Type II PLA<sub>2</sub> standard (lanes 1 and 8), was identified in homogenate (lane 2), post-dialysis supernatant (lane 3) and anti-MSA-treated supernatants (lanes 4 and 5) (Fig. 2c). This immunoreactive band was diminished by the first cycle of anti-Type II PLA<sub>2</sub> antibody treatment (lane 6) and absent after the second cycle of anti-Type II PLA<sub>2</sub> antibody treatment (lane 7). cPLA<sub>2</sub> immunoactivity was

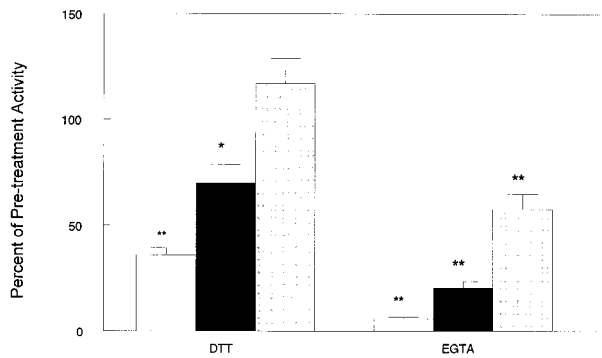
present throughout tissue processing and immunoaffinity batch extraction (Fig. 2b).

### Quantitative effects of immunoaffinity batch extraction

The quantitative effects of immunoaffinity batch extraction on Type II PLA<sub>2</sub> immunoactivity and total PLA<sub>2</sub> enzyme activity are presented in Fig. 3 as a percentage of activity present in the pretreatment sample. Treatment of placental extracts ( $n=3$ ) with Type II PLA<sub>2</sub> antibody coupled to agarose beads resulted in complete removal of Type II PLA<sub>2</sub> (i.e. not detected by ELISA) and a concomitant loss of  $82 \pm 1\%$  of PLA<sub>2</sub> enzymatic activity



**Figure 3** Immunoaffinity extraction of Type II PLA<sub>2</sub> from human term placental extracts ( $n=3$ ) using control monoclonal antibody-agarose conjugate (Anti-MSA) or anti-Type II PLA<sub>2</sub> conjugate (Anti-PLA<sub>2</sub>). Placental extracts were subjected to two cycles (1st and 2nd) of immunoaffinity extraction and assayed for enzymatic activity and Type II PLA<sub>2</sub> immunoactivity. Data are presented as percentage of pretreatment activity and represent the mean  $\pm$  S.E.M. ( $n=3$ ). nd, not detectable.



**Figure 4** Characterisation of residual PLA<sub>2</sub> enzymatic activity present in placental extracts after two cycles of immunoaffinity extraction. Residual PLA<sub>2</sub> enzymatic activity (solid bar) was assayed in the absence (control) or presence of inhibitors of secretory PLA<sub>2</sub> enzymatic activity: DTT (12.5 mM) or EGTA (12.5 mM). The effect of these treatments on recombinant Type II PLA<sub>2</sub> (open bar) and cPLA<sub>2</sub> (shaded bar) were also assessed. In addition, data are presented as a percentage of pretreatment activity ( $n=3$ ). Group means were compared with pretreatment values: \* $P<0.05$ , \*\* $P<0.01$ .

( $P<0.001$ ). Similar treatment of placental extracts with a control antibody (MSA) resulted in the recovery of  $107 \pm 1\%$  of enzymatic activity and  $106 \pm 3\%$  of Type II immunoactivity.

#### Characterisation of residual PLA<sub>2</sub> enzymatic activity

The effects of DTT and EGTA on PLA<sub>2</sub> enzymatic activity remaining after two cycles of immunoaffinity batch extraction are presented in Fig. 4. Comparative data (controls) for human recombinant Type II PLA<sub>2</sub> and cPLA<sub>2</sub> standards are also presented. Data are presented as percentage of pretreatment enzymatic activity ( $n=3$ ). Pretreatment placental extract, human recombinant Type II PLA<sub>2</sub> and residual PLA<sub>2</sub> enzymatic activity were significantly inhibited in the presence of reducing agent (12.5 mM DTT;  $P<0.05$ ). Pretreatment placental extract displayed the greatest sensitivity to DTT inhibition with  $89 \pm 3\%$  of activity inhibited ( $P<0.001$ ) followed by human recombinant Type II PLA<sub>2</sub> ( $64 \pm 1\%$ ,  $P<0.01$ ) and residual PLA<sub>2</sub> ( $30 \pm 9\%$ ,  $P<0.05$ ). cPLA<sub>2</sub> standard isolated from rabbit lung was unaffected by DTT treatment. The enzymatic activity of all preparations was suppressed by treatment with calcium-chelating agent (EGTA, 12.5 mM). Pretreatment placental extract and human recombinant Type II PLA<sub>2</sub> displayed the greatest sensitivity to EGTA inhibition with  $95 \pm 1\%$  and  $94 \pm 1\%$  of activity inhibited ( $P<0.01$ ), followed by residual PLA<sub>2</sub> ( $80 \pm 3\%$ ,  $P<0.01$ ) and cPLA<sub>2</sub> ( $42 \pm 7\%$ ,  $P<0.01$ ).

#### Discussion

Multiple isozymes of PLA<sub>2</sub> have been identified in human term placenta and may contribute to cell membrane

phospholipid metabolism and the formation of second messengers during pregnancy and at the time of labour and delivery (Rice 1996). To date, the contribution made by individual PLA<sub>2</sub> isozymes to PLA<sub>2</sub> enzymatic activity has not been established. Thus, the aim of this study was to establish the contribution made by Type II PLA<sub>2</sub> to total PLA<sub>2</sub> enzymatic activity present in human term placenta when assayed under *in vitro* conditions.

In this study, high ionic strength (HIS) extraction medium (i.e. 1 M NaCl) was used to facilitate the recovery of both secretory and cytosolic PLA<sub>2</sub> isozymes from placental homogenates. We have previously demonstrated that the use of such medium results in a 10- to 20-fold greater recovery of PLA<sub>2</sub> compared with the conventionally used low ionic strength extraction media (such as 0.16 M sucrose, Tris-buffered saline or PBS) (Aitken *et al.* 1993, Farrugia *et al.* 1993). Furthermore, HIS medium effectively dissociates calcium-dependent cPLA<sub>2</sub>-cell membrane binding (Clark *et al.* 1990), based on the observation that the inclusion of chelating agent (3 mM EGTA) in HIS medium does not result in increased recovery of PLA<sub>2</sub> activity (Farrugia *et al.* 1993). The presence of both secretory and cytosolic PLA<sub>2</sub> in HIS extracts of placenta was confirmed by Western blot analysis (Fig. 1). PLA<sub>2</sub> enzymatic activity present in HIS extracts was assayed under conditions optimal for both secretory and cytosolic PLA<sub>2</sub> (i.e. the presence of millimolar calcium concentrations, neutral-alkaline pH and an *sn*-2-arachidonylaminophospholipid substrate (Rice 1995)) and was consistent with previously reported values (Aitken *et al.* 1993, Farrugia *et al.* 1993).

The treatment of placental extracts with Type II PLA<sub>2</sub> monoclonal antibody coupled to agarose beads resulted in complete removal of irType II PLA<sub>2</sub>, as determined by ELISA. The specificity of the antibody used to remove irType II PLA<sub>2</sub> from placental extracts was confirmed by Western blot analysis. After SDS-PAGE fractionation of placental proteins, the antibody bound to a protein band that co-migrated with Type II PLA<sub>2</sub> standard, and no significant cross-reactivity with other placental proteins was observed (Fig. 1). Using this antibody, immunoaffinity extraction of Type II PLA<sub>2</sub> was associated with an 82% decrease in PLA<sub>2</sub> enzymatic activity (Fig. 3). No significant loss of immunoactivity and enzymatic activity was associated with parallel extractions in which a control monoclonal antibody (MSA) was used.

After the removal of Type II PLA<sub>2</sub> by two cycles of immunoaffinity batch extraction, residual PLA<sub>2</sub> enzymatic activity was still detectable. The presence of immunoreactive cPLA<sub>2</sub> in immunoaffinity-treated extracts was confirmed by Western blot analysis (Fig. 2), and thus this isozyme must contribute to the observed residual enzymatic activity. To characterise the residual activity further, the effects of known inhibitors of secretory PLA<sub>2</sub> isozymes were determined. The data obtained using DTT and EGTA treatment, while not unequivocal, are suggestive

that both cytoplasmic and secretory PLA<sub>2</sub> isozymes contribute to the residual activity. The residual activity displayed a response to inhibitors that fell between that of recombinant Type II PLA<sub>2</sub> and cPLA<sub>2</sub> (Fig. 4). It is not possible from the data obtained in this study to determine whether the secretory PLA<sub>2</sub> component of residual activity (i.e. DTT-sensitive) represents a non-Type II PLA<sub>2</sub> (e.g. Type IV PLA<sub>2</sub> which has been identified in human placenta; Freed *et al.* 1997) or the presence of Type II PLA<sub>2</sub> at a concentration that is below the limit of detection of the ELISA (5 ng/ml). The development of further immunological probes will be required to resolve this issue. The data obtained in this study, however, unequivocally established Type II PLA<sub>2</sub> as a quantitatively important PLA<sub>2</sub> isozyme present in human term placenta.

While the precise physiological role of placental Type II PLA<sub>2</sub> remains to be elucidated, this isozyme is released from term placenta (Farrugia *et al.* 1997) and is present in maternal plasma during pregnancy (Rice *et al.* 1992). The concentration of irType II PLA<sub>2</sub> in maternal plasma increases at the time of labour and delivery both at term and preterm. The release of PLA<sub>2</sub> from placenta (and possibly other gestational tissue, including amnion and choriondecidua; Aitken *et al.* 1993) may contribute to increased release of arachidonic acid and its subsequent conversion to uterotonic eicosanoids that may facilitate labour and delivery. The efficacy of exogenous PLA<sub>2</sub> and arachidonic acid in stimulating eicosanoid formation has been previously established (Gu & Rice 1991, Hara *et al.* 1991). In addition to a role in the formation of eicosanoids during pregnancy, secretory PLA<sub>2</sub> isozymes have been proposed as regulators of the cell-surface expression of aminophospholipids (Rice 1996). The cell-surface expression of aminophospholipids has been implicated in cell membrane fusion (Connor *et al.* 1989), the assembly of cell-surface clotting factors (Gilbert & Drinkwater 1993) and cell recognition (Herrmann & Devaux 1990). Type II PLA<sub>2</sub> displays a substrate preference for aminophospholipids and thus may play an important role in the placenta in regulating the composition of the exoplasmic leaflet of the cell membrane bilayer. By hydrolysing aminophospholipids and limiting the expression of negatively charged phospholipids on the cell surface, this isozyme may help to prevent clot formation (for example, in the intervillous space) and affect the rate of cytotrophoblast syncytial formation.

In summary, the data obtained in this study support the conclusion that Type II PLA<sub>2</sub> is quantitatively the primary PLA<sub>2</sub> isozyme present in human term placenta. Under the experimental conditions used in this study, Type II PLA<sub>2</sub> accounted for at least 80% of total PLA<sub>2</sub> enzymatic activity in the placenta. Although this isozyme has been demonstrated to be in abundance in placental homogenate, it is a secreted isozyme and active in the extracellular compartment. Once released, Type II PLA<sub>2</sub> may contribute to placental phospholipid metabolism and eicosanoid

formation. Within the intracellular compartment, cPLA<sub>2</sub> contributes to the metabolism of phospholipids and the liberation of fatty acid substrate for eicosanoid formation. The relative contributions of these pathways to eicosanoid formation remain to be established.

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