ELSEVIER

Biochimica et Biophysica Acta 1316 (1996) 183–190

BBA Biochimica et Biophysica Acta

Human group II phospholipase A_2 in normal and diseased intervertebral discs

Ken-ichiro Miyahara [a,*], Tetsuo Ishida [b], Sinsuke Hukuda [a], Kihachiro Horiike [b], Mitsuhiro Okamoto [c], Hiromasa Tojo [c]

[a] *Department of Orthopaedic Surgery, Shiga University of Medical Science, Seta, Ohtsu, Shiga 520-21, Japan*
[b] *Department of Biochemistry, Shiga University of Medical Science, Seta, Ohtsu, Shiga 520-21, Japan*
[c] *Department of Molecular Physiological Chemistry, Osaka University Medical School, 2-2 Yamadaoka, Suita 565, Japan*

Received 1 March 1996; accepted 27 March 1996

Abstract

We measured calcium-dependent phospholipase A_2 (PLA_2) activity and immunoreactive group II PLA_2 levels of 54 normal discs obtained from cadavers and 73 disc samples surgically obtained from patients with spinal disorders, including intervertebral disc herniations, spondylosis, and spondylolisthesis. Both cadaveric and surgical disc specimens contained about two-fold greater PLA_2 activity than the ileal mucosa, one of the richest sources of group II PLA_2. Discs of middle-aged cases had significantly higher activity than those of younger and elder cases. In cadaveric normal discs, calcium-dependent PLA_2 activity was significantly higher in females than in males. Annulus fibrosus and nucleus pulposus contained the same PLA_2 levels. In diseased discs, herniated fragments that had extruded or protruded out of the discs possessed lower activity than other parts of discs in the intervertebral space. Immunoreactive group II PLA_2 levels of intervertebral discs closely correlated with PLA_2 enzymatic activity. We purified a PLA_2 from human intervertebral disc to homogeneity to further identify the isozymic nature of discal PLA_2. Its NH_2-terminal amino acid sequence and molecular weight were identical to those of human group II PLA_2. Immunohistochemical analysis using a monoclonal anti-group II PLA_2 antibody showed that in both annulus fibrosus and nucleus pulposus chondrocytes contained intense group II PLA_2 immunoreactivity in their cytoplasm, and that the matrix contained no substantial immunoreactivity. These results suggest that group II PLA_2 in chondrocytes has important physiological roles in discal ordinary metabolism, maintaining discal homeostasis.

Keywords: Phospholipase A_2; Intervertebral Disc; Cartilage; Chondrocyte; Spine; (Human)

1. Introduction

Phospholipase A_2 (PLA_2) hydrolyzes the *sn*-2 fatty acyl ester bond of glycerophospholipids to produce free fatty acids and lysophospholipids. This enzyme plays important roles in remodeling membrane phospholipids, in regulating icosanoid biosynthesis, and in mediating inflammatory reactions [1,2].

Recent findings of high levels of PLA_2 activity in herniated nucleus pulposus suggested that PLA_2 within the nucleus pulposus can cause nerve injury and that activation of PLA_2 within the intervertebral disc contributes to the process of disc degeneration [3]. However, the precise roles played by PLA_2 in the pathogenesis of disc herniation remain to be clarified, because the properties and the origin of PLA_2 within the discs normal and diseased are unknown.

In this study we purified a PLA_2 from human intervertebral discs to homogeneity and confirmed that its N-terminal amino acid sequence was identical to human group II PLA_2. The location of group II PLA_2 in discs was immunohistochemically determined with specific monoclonal antibodies raised against human group II PLA_2. We measured PLA_2 activity and immunoreactive group II PLA_2 (IR-group II PLA_2) levels of uninflamed intervertebral disc samples obtained from normal fresh cadavers and

Abbreviations: PLA_2, phospholipase A_2; IR-group II PLA_2, immunoreactive group II phospholipase A_2; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; HPLC, high-performance liquid chromatography; ADAM, 9-anthryldiazomethane; SDS, Sodium dodecyl sulfate; JOA score, an evaluation score proposed by the Japanese Orthopaedic Association for spinal lesions; OPLL, ossification of posterior longitudinal ligament of cervical spine

* Corresponding author. Fax: +81 775 48 2254; Tel: +81 775 48 2252; e-mail: miyahara@belle.shiga-med.ac.jp

PII S0925-4439(96)00023-3

from patients with a variety of spinal disorders. We further compared PLA_2 levels of disc portions remaining in the original disc space with those of extrudedly and protrudedly herniated portions of discs.

2. Materials and methods

2.1. Disc materials

Human intervertebral discs ($n = 73$) were obtained from 45 patients, 26 females and 19 males, aged 12-80 years (mean 41.4), who underwent discectomy from the anterior or posterior approach (Table 1). Erythrocyte sedimentation rates, serum C-reactive protein concentration, and leukocyte counts of the patients were normal. Severity of clinical symptoms was assessed using respective evaluation scores for cervical and lumbar lesions proposed by the Japanese Orthopaedic Association (JOA score). To assess disc degeneration, magnetic resonance imaging (MRI) findings of the patients were evaluated using the grading scale of Eyre et al. [4]. After surgical removal, disc samples were immediately frozen and stored at −80°C.

Since it is ethically impossible to obtain normal disc samples from patients and healthy subjects, intervertebral discs ($n = 54$) were obtained from 10 fresh human cadavers, aged 19–73 years (mean 46.2), which had been dissected at the Osaka Medical Examiner's Office. None of the deceased had an obvious history of spinal disorders and autopsy confirmed that they had not suffered from any inflammatory disorders. A portion of each disc was fixed in formalin and stained with hematoxylin-eosin and toluidine blue. All of the discs were histologically normal with some age-dependent changes [5]. The results of preliminary experiments showed that incubation of cadaveric disc homogenates did not affect PLA_2 activity for at least 8 h. Hence, we used cadaveric discs as control specimens. Annuli and nuclei of C6/7, L1/2 and L4/5 interval discs were removed and stored at −80°C.

Table 1
Intervertebral disc samples obtained from patients with various spinal disorders

Spinal disorders	Number of cases	Mean age (range)	Number of discs [a]
Intervertebral disc herniation	28	39 (13–70)	37
Spondylosis	5	53 (35–80)	12
Spondylolisthesis	4	51 (45–59)	5
Idiopathic scoliosis	2	12, 16	7
OPLL[b]	2	44, 75	5
Traumatic fracture/dislocation	2	21, 23	3
Neurenteric cyst	1	48	3
Metastatic spinal tumor	1	59	1
Total number	45		73

[a] In several cases, more than two disc samples were obtained from one patient.
[b] Ossification of posterior longitudinal ligament of cervical spine.

2.2. Assay for PLA_2 activity

Each disc sample (40–80 mg) was thawed at room temperature and then homogenized in 40 vol. of 25 mM Hepes-HCl buffer (pH 7.4) on ice with a glass homogenizer. An equal volume of 2 M KBr was added to the homogenate. After 30-min incubation at 4°C under continuous stirring, the mixture was centrifuged at $15000 \times g$ for 10 min at 4°C. An aliquot of the supernatant (2 μl) was used for the assay. PLA_2 activity was determined as reported previously [6,7]. Briefly, the assay mixture (50 μl) contained 0.8 mM 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (POPG, Avanti Polar Lipids), 5 mM cholate, 0.1 M Tris-HCl (pH 8.5), 0.1 M NaCl, 5 mM $CaCl_2$, and the enzyme. The reaction was stopped by adding 200 μl of the Dole's reagent, and then fatty acids were extracted by the method of Dole and Meinertz [8]. Heptadecanoic acid (5 nmol) (Nacalai Tesque, Kyoto) was used as an internal standard. Extracted fatty acids were labeled with 9-anthryldiazomethane (Funakoshi Co., Tokyo), and then each of the derivatized fatty acids was quantitated by reverse-phase high-performance liquid chromatography (HPLC) with UV detection at 254 nm. Calcium-dependent PLA_2 activity was estimated as the difference between the activity assayed in the presence of 5 mM $CaCl_2$ and that in the presence of 10 mM EDTA. Protein concentrations were determined with bicinchoninic acid protein assay reagent (Pierce).

2.3. Assay for immunoreactive group II PLA_2

IR-group II PLA_2 in the KBr extracts from intervertebral discs was measured by a sensitive radioimmunoassay kit specific to human group II PLA_2, using a monoclonal antibody against human splenic group II PLA_2 (donated by the Diagnostic Science Department, Shionogi and Co., Settsu, Osaka) [9].

2.4. Purification of PLA_2 from human intervertebral discs

Human intervertebral disc (5.4 g wet wt.) was cut into thin slices and then homogenized in 70 ml of 10 mM Tris-HCl (pH 7.4) with a glass homogenizer. After 20 min of incubation on ice under continuous stirring, the homogenate was centrifuged at $20800 \times g$ for 20 min at 4°C. The pellet was homogenized in 70 ml of 10 mM Tris-HCl (pH 7.4) containing 1 M KBr and kept on ice under continuous stirring for 30 min to extract PLA_2 activity. The homogenate was centrifuged at $20800 \times g$ for 20 min at 4°C. The supernatant (KBr extract) was diluted 10-fold with 10 mM Tris-HCl (pH 7.4), and applied to a SP Sepharose column (4 × 3 cm, Pharmacia Biotech) pre-equilibrated with the same buffer. The column was washed with 150 ml of 10 mM Tris-HCl (pH 7.4) containing 0.15 M NaCl, and then PLA_2 activity was eluted with 10 mM Tris-HCl (pH 7.4) containing 1 M NaCl and 0.1% Triton

X-100. The eluent (SP Sepharose-1) was diluted 10-fold with 10 mM Tris-HCl (pH 7.4) and applied again to a SP Sepharose column (2 × 1.5 cm) pre-equilibrated with 10 mM Tris-HCl (pH 7.4) containing 0.1% Triton X-100. After the column was washed with the same buffer, PLA_2 activity was eluted with 10 mM Tris-HCl (pH 7.4) containing 1 M NaCl and 0.1% Triton X-100. The eluent was further concentrated to about 1.8 ml with a Microcon 30 microconcentrator (Amicon). Aliquots (100–200 μl) of the concentrated PLA_2 solution were repeatedly (15 times) injected to a TSK gel $G2000SW_{XL}$ (7.8 × 150 mm, Tosoh) pre-equilibrated with 10 mM Tris-HCl (pH 7.4) containing 0.3 M NaCl and 0.1% Triton X-100. Elution was performed with the same buffer at a flow rate of 0.5 ml/min. The absorbance at 295 nm was continuously monitored. The pooled PLA_2-active fractions (Gel filtration) were concentrated using an SP Sepharose column (1 × 1 cm), as described above. The resultant solution (20 ml, SP Sepharose-2) was subjected to a Cosmosil 5C4-300 column (4.6 × 50 mm, Nacalai Tesque) pre-equilibrated with 0.1% trifluoroacetic acid (TFA) in water. The column temperature was maintained at 20°C. Elution was performed with the linear gradient of acetonitrile in 0.1% TFA: 0–25% in 5 min, 25–35% in 40 min, and then 35–100% in 5 min at the flow rate of 0.3 ml/min.

2.5. Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed on a 15% gel according to the method of Laemmli [10]. Proteins were stained with 0.25% Coomassie Brilliant Blue R-250 in 45% methanol-9% acetic acid. The marker proteins were purchased from Boehringer Mannheim.

2.6. NH_2-terminal amino acid sequence and molecular weight determination

NH_2-terminal amino acid sequence analysis was performed by an Applied Biosystem 473A and a 120A PTH analyzer. Matrix-assisted laser desorption mass spectra were obtained on a Shimadzu/Kratos laser ionization time-of-flight (TOF) mass spectrometer (KOMPACT MALDI III). α-Cyano-4-hydroxycinnamic acid was used as a matrix-forming material and myoglobin was used for mass calibration.

2.7. Immunohistochemistry

Formalin-fixed and paraffin-embedded sections of disc specimens that were obtained at autopsy and surgery were reacted with mouse monoclonal anti-group II PLA_2 antibody. The primary immunoreaction was visualized using an ABC kit (Vector Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions [11]. For control, the primary antiserum was omitted or was replaced by anti-group I (pancreatic) PLA_2 antibody. Cartilage of the femoral condyle from a 59-yr-old female suffering from rheumatoid arthritis was also examined to compare stainability of articular cartilage with that of disc cartilage.

2.8. Statistical analysis

Data were expressed as mean ± standard deviation (S.D.). Data of PLA_2 activity and IR-group II PLA_2 levels were analyzed by Mann-Whitney test. Regression analysis was used to determine the relationships between PLA_2 activity and IR-group II PLA_2 levels, MRI grades or JOA scores. Results of $P < 0.05$ were considered significant.

3. Results

3.1. Characterization of PLA_2 purified from human intervertebral discs

With minor modifications of the HPLC method [6,7,12], a PLA_2 (14 mg) was purified to homogeneity from human intervertebral discs (5.4 g wet wt.) with the overall recovery of 7.7% and about 150-fold purification (Table 2). At each chromatographic step during purification, PLA_2 activity was eluted as a single peak (data not shown). The specific activity of 420 μmol/min per mg was obtained with the mixed micelles of POPG and cholate as the substrate, and is similar to that of group II PLA_2 purified from human ileal mucosa [12]. The purified enzyme migrated as a single band with an apparent molecular mass of 14.9 kDa on SDS-polyacrylamide gel (data not shown). The molecular mass was determined to be 13 888 Da by mass spectrometry. The sequence of the NH_2-terminal 25 residues of human intervertebral disc PLA_2 was determined to be NLVNFHRMIKLTTGKEAALSYGFYG and

Table 2
Purification of PLA_2 from human intervertebral disc

Step	Total vol. (ml)	Total protein (mg)	Total act. (μmol/min)	Specific act. (μmol/min per mg protein)	Yields (%)	Purification (-fold)
KBr extract	74.0	26.3	75.5	2.87	100	1
SP Sepharose-1	50.0	6.0	72.5	12.1	96	4.2
Gel filtration	11.0	0.572	24.9	43.5	33	15.2
SP Sepharose-2	2.0	0.394	17.4	44.2	23	15.4
HPLC	0.12	0.014	5.8	420	7.7	146

identical to that of human group II PLA_2 purified from spleen [13].

3.2. PLA_2 activity and immunoreactive group II PLA_2 levels in intervertebral discs

Table 1 summerizes 73 disc samples obtained from patients with spinal disorders. The disc samples ($n = 19$) obtained from patients with idiopathic scoliosis, OPLL, trauma, neurenteric cyst, and metastatic tumor were apparently normal.

All examined discs contained PLA_2 activity and IR-group II PLA_2 levels greater than those of other human group II PLA_2-rich organs, e.g., the ileum mucosa (51 nmol/min/mg protein) [14] (Fig. 1). Discal PLA_2 activity correlated with IR-group II PLA_2 levels ($r = 0.887$, $P < 0.0001$).

PLA_2 activity in surgical and cadaveric specimens were 87.3 ± 68.3 nmol/min/mg protein ($n = 73$) and 101 ± 71 nmol/min/mg protein ($n = 54$), respectively; and IR-group II PLA_2 levels in surgical and cadaveric specimens were 878 ± 908 ng/mg protein and 1005 ± 857 ng/mg protein, respectively. In both PLA_2 activity and IR-group II PLA_2 levels, there was no significant difference between discs obtained from patients and those from cadavers.

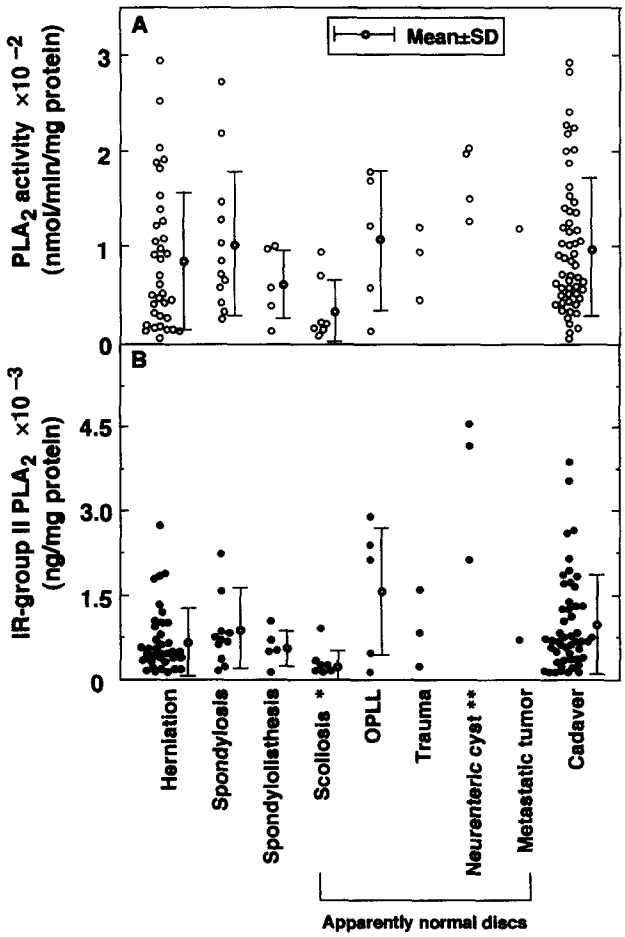

Fig. 1. PLA_2 activity (A) and IR-group II PLA_2 levels (B) in various disc conditions. Discs were obtained from patients with a variety of spinal diseases indicated in Table 1 ($n = 73$) and from cadavers without spinal and inflammatory disorders ($n = 54$). Discs obtained from patients with scoliosis, OPLL, trauma, neurenteric cyst and metastatic tumor showed apparently normal appearance. *, $P < 0.01$; **, $P < 0.05$, compared with normal cadaveric discs.

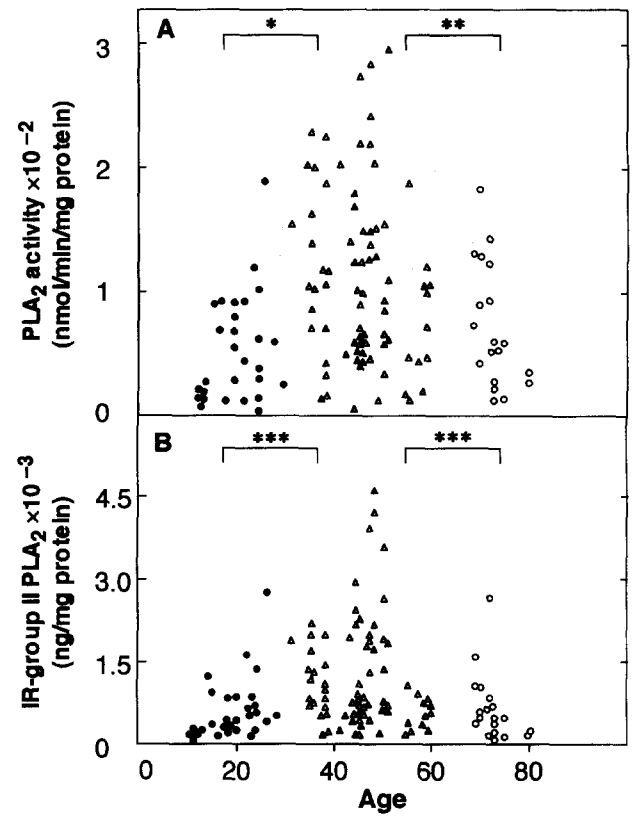

Fig. 2. Age-dependence of PLA_2 activity (A) and IR-group II PLA_2 levels (B). Patients and cadavers were divided into three groups according to age: ●, aged 12–29 yr; △, aged 30–59 yr; ○, ≥ 60 yr old. *: $P < 0.0002$, **: $P < 0.03$, ***: $P < 0.002$.

As shown in Fig. 2, discs of the middle-aged (30–59 years old, 110 ± 70 nmol/min per mg protein) had significantly higher activity than those of the second and third decades (12–29 years old, 60.3 ± 55.0 nmol/min per mg protein, $P < 0.0002$) and those of elders (≥ 60 years old, 70.3 ± 49.2 nmol/min per mg protein, $P < 0.03$). Similarly, discal IR-group II PLA_2 levels in the middle-aged (1153 ± 981 ng/mg protein) were significantly greater than those of the second and third decades (570 ± 564 ng/mg protein, $P < 0.002$) and those of elders (513 ± 386 ng/mg protein, $P < 0.002$).

In cadaveric normal discs, PLA_2 activity ($P < 0.04$) and IR-group II PLA_2 levels ($P < 0.04$) were significantly higher in females (118 ± 78 nmol/min/mg protein, and 1255 ± 987 ng/mg protein, respectively) than in males (76.7 ± 52.4 nmol/min per mg protein, and 637 ± 429 ng/mg protein, respectively) (Fig. 3). Ages of female group (19–72) matched those of male groups (24–73). No

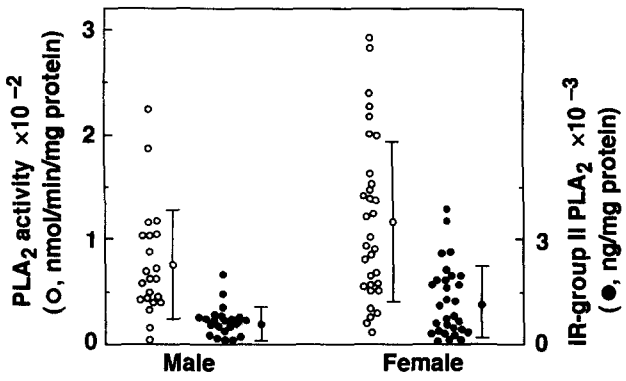

Fig. 3. Sexual disparity of PLA_2 activity (○) and IR-group II PLA_2 levels (●) in all cadaveric normal discs. ($P < 0.04$).

significant differences were found between PLA_2 activities in annulus fibrosus (109.8 ± 81.7 nmol/min per mg protein) and those in nucleus pulposus (92.3 ± 58.5 nmol/min per mg protein) (Fig. 4). The PLA_2 activity in intervertebral disc had no correlation with spinal levels.

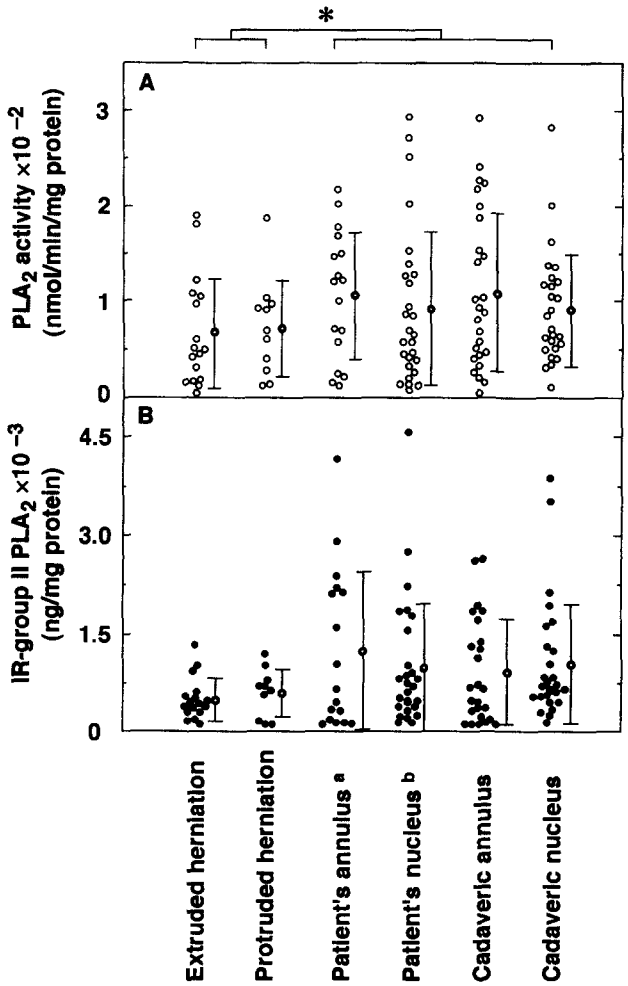

Fig. 4. PLA_2 activity (A) and IR-group II PLA_2 levels (B) in various disc samples. [a], portion of annulus fibrosus of surgical specimens other than herniated disc fragments. [b], portion of nucleus pulposus of them. *, $P < 0.05$.

3.3. PLA_2 activity and immunoreactive group II PLA_2 levels in diseased discs

In surgical specimens, the PLA_2 activity and IR-group II PLA_2 levels tended to be higher in females than in males, as recognized in cadaveric specimens, but there was no statistical significance. Both PLA_2 activity and IR-group II PLA_2 levels did not correlate with degenerative grades of MRI findings of discs or with the clinical severity as assessed by JOA scores (data not shown). Both extruded and protruded portions of herniated discs [15] had lower PLA_2 activity ($P < 0.05$) and IR-group II PLA_2 levels ($P < 0.05$) than the disc materials that remained at their original site. Ages of patients with disc herniations matched those of patients with other spinal disorders, and those of cadavers (Fig. 4).

Of all discs examined, thoracic disc materials surgically obtained from two patients with idiopathic scoliosis, aged 12 and 16, had significantly low PLA_2 activity and IR-group II PLA_2 levels ($P < 0.01$). On the contrary, cervical disc materials obtained from a 48-year-old female suffering from a neurenteric cyst in the spinal canal had significantly high activity and IR-group II PLA_2 levels ($P < 0.05$) (Fig. 1).

3.4. Immunohistochemistry

All of the chondrocytes examined in both annulus fibrosus and nucleus pulposus exhibit group II PLA_2 immunoreactivity in their cytoplasm (Fig. 5). Unlike articular chondrocytes (Fig. 6), the chondrocyte lacunae and the matrix of discs had no IR-group II PLA_2. Control sections using anti-group I (pancreatic) PLA_2 antibody were not stained at all.

The staining patterns of articular cartilage differ from those of disc cartilage. There was a large amount of IR-group II PLA_2 in the deep layer adjacent to subchondral bone, while there was its small amount in the superficial layer near the joint space. In agreement with previous results [16], the chondrocyte lacunae and surrounding cartilage matrix, as well as the cytoplasm of the chondrocytes, contained IR-group II PLA_2 (Fig. 6).

4. Discussion

In this study we found high PLA_2 activity and IR-group II PLA_2 levels in all intervertebral discs obtained from patients with disc herniations and other spinal disorders, and normal discs obtained from cadavers. Mean enzymatic activity and IR-group II PLA_2 levels per the protein concentration in the intervertebral discs were greater than that found in normal terminal ileal mucosa, one of the richest source of group II PLA_2. In comparison with activity in various rat tissues assayed by the same method, human intervertebral discs showed a level of activity as

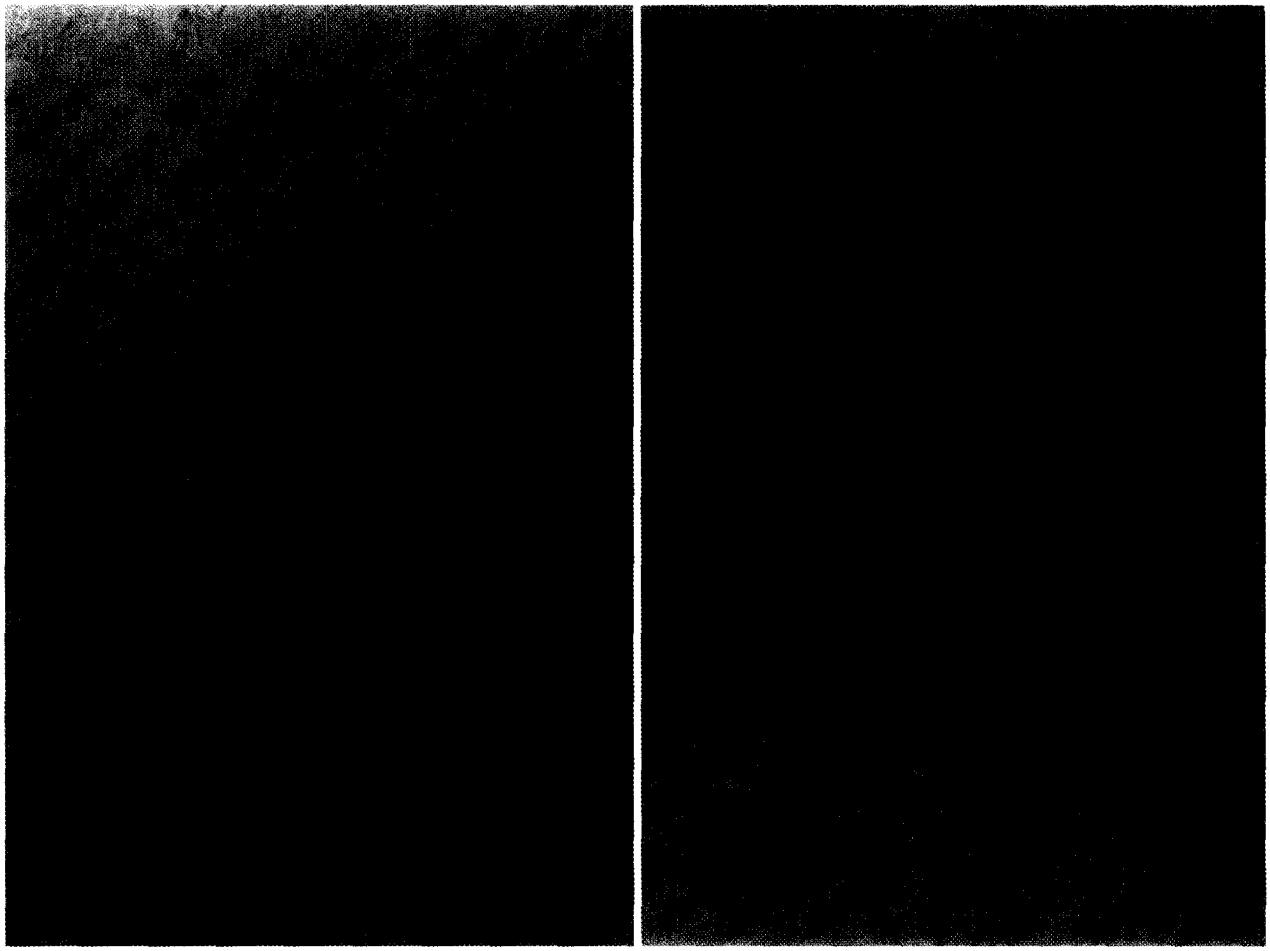

Fig. 5

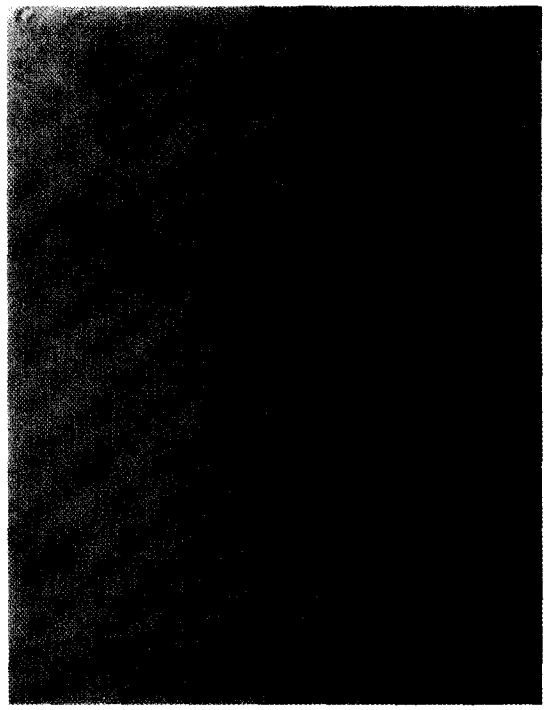

Fig. 6

high as in rat pancreas or spleen [7]. Mean IR-group II PLA_2 levels was 25 times higher than those of inflamed colonic mucosa of patients with Crohn's disease and ulcerative colitis [14]. It is surprising that normal intervertebral discs with sparse cellularity showed higher IR-group II PLA_2 levels than inflamed mucosa having much higher cellularity. Since the discs contain abundant extracellular proteins and have greater protein contents than colonic and ileal mucosas, the amount of group II PLA_2 per a cell in the discs is much greater than those of intestinal mucosal cells.

We purified a PLA_2 with a molecular mass of 13888 Da from human intervertebral discs, and the sequence of its NH_2-terminal 25 amino acid residues was identical to that of human group II PLA_2 purified from spleen [13]. At all the chromatographic steps during purification, PLA_2 activity was eluted as a single peak. The discal PLA_2 activity closely correlated with IR-group II PLA_2 levels. These results indicate that group II PLA_2 is responsible for measurable calcium-dependent PLA_2 activity in intervertebral discs.

IR-group II PLA_2 was localized in the cytoplasm of chondrocytes in both annulus fibrosus and nucleus pulposus. All chondrocytes in dises were uniformly stained and the chondrocyte lacunae and disc matrix had no group II PLA_2 immunoreactivity. These results suggest that the PLA_2 is stored in and secreted from chondrocytes in response to stimulations.

Recently articular cartilage was found to contain large quantities of group II PLA_2 in both osteoarthritis and rheumatoid arthritis [16–21]. A previous immunohistochemical study [16] showed that in articular, laryngeal and auricular cartilage the peripheral areas of the chondrocyte lacunae and surrounding cartilage matrix contain IR-group II PLA_2 and that there were two distinct populations of PLA_2-containing and PLA_2-deficient chondrocytes. We confirmed these findings in this study, and demonstrated that the localization of group II PLA_2 in intervertebral discs and the articular cartilage is quite different. This may be interpreted that chondrocytes in the disc store the enzyme in the secretary granule, like intestinal Paneth cells (Tojo et al. unpublished results), whereas chondrocytes in the articular cartilage are continuously releasing the enzyme into the extracellular matrix after its synthesis.

Chondrocytes in the deep layer of articular cartilage adjacent to subchondral bone had a larger amount of IR-group II PLA_2 than in the superficial layer near the joint space. These results coincide with the fact that deep layers of articular cartilage show higher PLA_2 activity than superficial ones in rheumatoid arthritis and osteoarthritis [19], and that cultured chondrocytes obtained from the deep layers of the cartilage released more PLA_2 than those obtained from the superficial layers [20].

Discs of middle-aged patients and cadavers had significantly high PLA_2 activity and IR-group II PLA_2 levels as compared with those of younger and elder cases. For instance, discs of two young girls with idiopathic scoliosis had significantly low PLA_2 activity and IR-group II PLA_2 levels, while discs of a middle-aged patient with neurenteric cyst had significantly high PLA_2 activity and IR-group II PLA_2 levels. Since the histological appearance and MRI findings of these cases were normal, the measured difference in the activity and IR-group II PLA_2 levels may be due to physiological age-dependent changes in expression levels of the enzyme. In this context, Recklies and White reported that PLA_2 activity was not detectable in cartilage from newborns [21]. These findings suggest that group II PLA_2 develops postneonatally, that is, its amounts in discs gradually increase during adolescence, reach the peak of synthesis at middle age, and then decrease with aging. A decrease in the number of viable chondrocytes in senescent discs [5] may cause reduction of PLA_2 activity. An immunohistochemical study [22], however, suggests that the fetal gastric mucosa contained a large amount of IR-group II PLA_2, but not the adult mucosa.

Cadaveric discs of females had higher PLA_2 activity and IR-group II PLA_2 levels than those of males, although serum IR-group II PLA_2 levels did not depend on sex (H. Tojo, unpublished results). It remains to be clarified whether or not the expression of group II PLA_2 in intervertebral discs is hormonally regulated.

The nucleus pulposus consists of gel-like materials that are rich in proteoglycans, whereas the annulus fibrosus is mainly composed of collagen fibers. Since there was no difference in the PLA_2 activity and IR-group II PLA_2 levels between nucleus pulposus and annulus fibrosus, the difference in local environments of cartilage matrix may not affect the degree of expression of group II PLA_2 in the chondrocytes.

Herniated disc fragments had lower PLA_2 activity and IR-group II PLA_2 levels than disc materials remaining within the site. When a disc fragment has herniated out of

Fig. 5. Immunohistochemical localization of group II PLA_2 in the intervertebral disc in normal cadaveric intervertebral disc of a female aged 47, using anti-human group II PLA_2 monoclonal antibody. A. Despite sparse cellularity, all of the chondrocytes were intensely stained with the antibody. B. High power magnification of the part indicated by an arrow in A. The cytoplasm of disc chondrocytes contained IR-group II PLA_2. The chondrocyte lacunae and the matrix of discs had no immunoreactive PLA_2.

Fig. 6. Staining in a femoral condyle of a female aged 59 suffering from rheumatoid arthritis, using anti-human group II PLA_2 monoclonal antibody. Articular cartilage showed varying degrees of stainability to the group II PLA_2 antibody. The chondrocyte lacunae and surrounding cartilage matrix, as well as the cytoplasm of the chondrocytes, contained IR-group II PLA_2. There was a marked amount of IR-group II PLA_2 in the deep layer adjacent to subchondral bone (top), while there was its small amount in the superficial layer near the joint space (bottom).

its original site, the ordinary nutritional pathway of chondrocytes may be obstructed, leading to decreasing production of PLA_2. This result is compatible with the fact that the amounts of prostaglandin released from sequestrated discs as measured by radioimmunoassay, were very low, compared with those from lumbar facet cartilage and bone [23]. Alternatively, there is a possibility that certain stimuli in herniated discs increase the secretion of group II PLA_2, leading to depleting the enzyme store.

Prostaglandins and leukotrienes stimulate neural elements and cause pain [24,25], and, in addition, activation of PLA_2 produces mechanical hyperalgesia [26]. Thus, PLA_2 activity in discs seems to affect the severity of symptoms. However, in this study, the activity did not correlate with clinical features of patients assessed by JOA scores. The PLA_2 activity measured by the present method may not reflect PLA_2 activity around nerves in the vicinity of herniated discs, or it may be unrelated to icosanoid production. Since herniated discs contain low levels of PLA_2 and prostaglandins, immunologic reactions might be more responsible for pathophysiology of radiculopathy [23].

PLA_2 liberates arachidonic acid from membrane phospholipids at the site of inflammation, and extracellular group II PLA_2 expression has been linked to the potentiation and perpetuation of inflammatory reactions [27]. However, because morphologically normal cadaveric discs had the same group II PLA_2 levels and activity as surgical specimens, we conclude that group II PLA_2 is constitutively produced and plays important physiological roles in discal ordinary metabolism to maintain cartilage homeostasis. In the metabolism of chondrocytes, prostaglandins are implicated in the regulation of cartilage differentiation: prostaglandin E_2 stimulates the development of chick limb and chondrogenesis [28], and inhibitors of prostaglandin synthesis inhibit chondrogenesis in limb cells in vitro [29]. Mineralization of cartilage adjacent to the growth zone is associated with elevated levels of alkaline phosphatase and PLA_2 of matrix vesicles in cartilage [30]. These facts suggest that PLA_2 may participate in diverse metabolic processes. Further investigations are necessary to elucidate the function of group II PLA_2 in the metabolism of intervertebral discs.

Acknowledgements

We would like to thank Prof. K. Nishi and Dr. A. Kohno (Department of Legal Medicine of Shiga University of Medical Science and the Osaka Medical Examiner's Office) for providing the cadaveric discs used in this study. We would also like to thank Dr. M. Kakiuchi (Osaka Police Hospital) for providing surgical specimens of intervertebral disc herniations, and Dr. K. Otani and Dr. M. Saito (National Murayama Hospital) for providing discs of scoliosis patients. We wish to thank Dr. S. Ohnishi (Shimadzu Analytical Applications Laboratory) for his cooperation in the use of the mass spectrometer.

References

[1] Glaser, K.B., Mobilio, D., Chang, J.Y. and Senko, N. (1993) Trends Pharmacol. Sci. 14, 92–98.
[2] Pruzanski, W. and Vadas, P. (1991) Immunol. Today 12, 143–146.
[3] Saal, J.S., Franson, R.C., Dobrow, R., Saal, J.A., White, A.H. and Goldthwaite, N. (1990) Spine 15, 674–678.
[4] Eyre, D., Benya, P. and Buckwalter, J. (1989) in New Perspectives On Low Back Pain (Frymoyer, J.W. and Gordon, S.L., eds.), pp. 147–207, American Academy of Orthopaedic Surgeons, Park Ridge, Illinois.
[5] Buckwalter, J.A. (1995) Spine 20, 1307–1314.
[6] Tojo, H., Ono, T. and Okamoto, M. (1991) Methods Enzymol. 197, 390–399.
[7] Tojo, H., Ono, T. and Okamoto, M. (1993) J. Lipid Res. 34, 837–844.
[8] Dole, V.P. and Meinertz, H. (1960) J. Biol. Chem. 235, 2595–2599.
[9] Matsuda, Y., Ogawa, M., Sakamoto, K., Yamashita, S., Kanda, A., Kohno, M., Yoshida, N., Nishijima, J., Murata, A. and Mori, T. (1991) Enzyme 45, 200–208.
[10] Laemmli, U.K. (1970) Nature 227, 680–685.
[11] Hsu, S-M., Raine, L. and Fanger, H. (1981) J. Histochem. Cytochem. 29, 577–580.
[12] Minami, T., Tojo, H., Shinomura, Y., Matsuzawa, Y. and Okamoto, M. (1993) Biochim. Biophys. Acta 1170, 125–130.
[13] Kanda, A., Ono, T., Yoshida, N., Tojo, H. and Okamoto, M. (1989) Biochem. Biophys. Res. Commun. 163, 42–48.
[14] Minami, T., Tojo, H., Shinomura, Y., Matsuzawa, Y. and Okamoto, M. (1994) Gut 35, 1593–1598.
[15] American academy of orthopaedic surgeons (1981) A glossary on spinal terminology, Chicago.
[16] Nevalainen, T.J., Märki, F., Kortesuo, P.T., Grütter, M.G., Marco, S.D. and Schmitz, A. (1993) J. Rheumatol. 20, 325–330.
[17] Stevens, T.M., Chin, J.E., McGowan, M., Giannaras, J. and Kerr, J.S. (1989) Agent Action 27, 385–387.
[18] Vignon, E., Mathieu, P., Louisot, P., Vilamitjana, J., Harmand, M.F. and Richard, M. (1989) J. Rheumatol. 16 (S18), 35–38.
[19] Pruzanski, W., Bogoch, E., Stefanski, E., Wloch, M. and Vadas, P. (1991) Life Science 48, 2457–2462.
[20] Pruzanski, W., Bogoch, E., Stefanski, E., Wloch, M. and Vadas, P. (1991) J. Rheumatol. 17, 1386–1391.
[21] Recklies, A.D. and White, C. (1991) Arthritis Rheum. 34, 1106–1115.
[22] Kiyohara, H., Egami, H., Shibata, Y., Murata, K., Ohshima, S. and Ogawa, M. (1992) J. Histochem. 11, 1659–1664.
[23] Willburger, R.E. and Wittenberg, R.H. (1994) Spine 19, 2068–2070.
[24] Levine, J.D., Lau, W., Kiniat, G. and Goetzl, E.J. (1984) Science 225, 743–745.
[25] Pateromichelakis, S. and Rood, J.P. (1981) Experientia 37, 282–284.
[26] Meller, S.T. and Gebhart, G.F. (1994) Drugs 47, 10–20.
[27] Vadas, P., Browning, J., Edelson, J. and Pruzanski, W. (1993) J. Lipid Mediators 8, 1–30.
[28] Kosher, R.A. and Walker, K.H. (1983) Exp. Cell Res. 145, 145–153.
[29] Chepenik, K.P., Ho, W.C., Waite, B.M. and Parker, C.L. (1984) Calcif. Tissue Int. 36, 175–181.
[30] Yang, F., Schwartz, Z., Swain, L.D., Lee, C.C., Bowman, B.H. and Boyan, B.H. (1991) Bone 12, 7–15.