

FOURTEEN kilodalton phospholipase A₂ molecules (PLA₂) are classified into two groups, I- and II-PLA₂, and only the latter has been considered to play a pathogenetic role in various forms of tissue inflammation. Previously we demonstrated high PLA₂ activity in gingival crevicular fluid (GCF) of patients with periodontal disease, without determining the group of the enzyme involved. In this study, the activity, groups and levels of enzyme in gingiva taken from 13 sites of periodontal disease were determined using both biochemical and radioimmunological methods. A linear correlation between the activity and the level of II-PLA₂ was observed. No I-PLA₂ was found in any of the samples tested. These data suggest that the PLA₂ activity found in the GCF of patients with periodontal disease does not belong to the I-PLA₂, but to the II-PLA₂ group.

Key words: Disease activity, Periodontal disease, Phospholipase A₂

Group II phospholipase A₂ in human gingiva with periodontal disease

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Introduction

Intracellular phospholipase A₂ (PLA₂; EC 3.1.1.4) catalyses the hydrolysis of the ester bound at the *sn*-2 position of glycerophospholipids and is a key enzyme in the production of potent inflammatory mediators, including prostaglandins, leukotrienes and platelet activating factor. Recently, it has been demonstrated that mammalian PLA₂ can be classified into at least three groups on the basis of their primary structure.¹ Two of these groups are designated I- and II-PLA₂, and are found in pancreatic juice and a variety of tissues other than pancreas, respectively. Both groups have a molecular mass of 14 kDa.² The third group is present in the cytosolic fraction of various tissues and has a molecular weight of 85 kDa.³ Among these enzyme groups, only II-PLA₂ has been considered to play an important role in the process of inflammation, for example, in rheumatoid arthritis⁴ or inflammatory bowel disease.⁵ In our previous studies, it was demonstrated that rat gingival cells had II-PLA₂ activity and that this activity was induced by the inflammatory cytokines interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α), but inhibited by the anti-inflammatory cytokine transforming growth factor- β (TGF- β).^{6,7} In addition, we also demonstrated PLA₂ activity in human gingival crevicular fluid (GCF) from patients with periodontal disease and indicated that this activity appeared to reflect periodontal disease conditions (active or resting), suggesting that its potential usefulness as a marker for the assessment of disease activity.⁸ However, the maximum volume of GCF was

too small to determine whether the activity if contained belonged to the II-PLA₂ group, even with the sensitive method we employed. Since GCF contains infiltrates from adjacent gingival tissue that enter the gingival crevice, the enzyme activity in GCF reflects that in the gingival tissue.

To clarify the type of PLA₂ present in GCF from patients with periodontal disease, the activity and level of PLA₂ were measured in the gingival tissue using both biochemical and radioimmunological assay systems, respectively.

Materials and Methods

Gingival tissue: Gingival tissues from seven patients (mean age 42.0 \pm 2.2 years) who had at least five teeth with a pocket depth of more than 6 mm were used. The depth of the periodontal pocket (gingival crevice), which usually indicates the degree of periodontal tissue destruction, and its measurement is considered to be the most reliable tool for the diagnosis and assessment of periodontal disease. A tooth with healthy periodontal tissue has a pocket depth of less than 2 mm, whereas a tooth with a periodontal pocket depth of more than 4 mm is considered to be affected by periodontal disease. None of the patients were receiving or had previously received any medication or periodontal treatment before the study.

Gingival tissue preparation: Inflammatory gingival tissues were taken from 13 sites in the seven patients at the time of periodontal surgical therapy. The

excised tissues were rinsed thoroughly with ice-cold isotonic solution and weighed. Then they were minced and homogenized in 100 mM Tris-HCl (pH 7.4) using a glass homogenizer at 2 000 rpm for 30 s, followed by centrifugation at 20 000 \times g. An aliquot of each supernatant was used as the enzyme source for either biochemical determination of PLA₂ activity or radioimmunological PLA₂ assay.

Biochemical assay for PLA₂ activity: Phospholipase A₂ activity was determined according to the method described by Tojo *et al.*⁹ using 0.8 mM 1-palmitoyl-2 oleoyl-*sn*-glycero-3-phosphoglycerol as a substrate in the presence of 5 mM cholate. Fatty acids released by PLA₂ were labelled with 9-anthryldiazomethane and then separated by high-performance liquid chromatography column, followed by quantitation using marganic acid as an internal standard. Calcium-dependent PLA₂ was estimated as the difference between the activity assayed in the presence of 5 mM CaCl₂ and that in the presence of 10 mM ethylenediaminetetraacetate.

Radioimmunoassay for I- and II-PLA₂: The level of I- and II-PLA₂ in the supernatant was measured using a radioimmunoassay kit (Shionogi Pharmaceutical Ltd, Osaka, Japan) and a monoclonal antibody against human pancreatic group I- or splenic II-PLA₂ as described elsewhere.¹⁰

Results and Discussion

Figure 1 shows the sensitivity of the RIA used in this study and representative II-PLA₂ levels as a function of wet weight of gingival tissue. As can be seen, a minimum gingival tissue wet weight of about 1 mg was enough for detection of II-PLA₂. The level of II-PLA₂ increased with gingival wet weight over a range of 1 to 25 mg in a dose-dependent manner. It was not possible to determine whether the activity belonged to I- or II-PLA₂ in GCF, because usually the volume of GCF is less than 3 μ l, which is insufficient for RIA. However, the results suggested that the PLA₂ activity detected in GCF was classifiable by examining biopsy samples of gingival tissue. It would be acceptable and meaningful not only for periodontologists but also for patients for about 1 mg wet weight of gingiva to be taken as a biopsy sample to obtain precise and predictable information on the diseased sites.

Table 1 shows the levels of PLA₂ determined by the RIA in each supernatant of gingival tissue from 13 different sites. The mean level of II-PLA₂ was 83.7 pg/mg wet weight, with wide ranges in the enzyme level from 5 to 216 pg/mg wet weight. This wide range of values was not surprising, because our previous data showed that PLA₂ activity was well correlated only with periodontal disease activity, and not with the degree of destruction caused by the previous disease

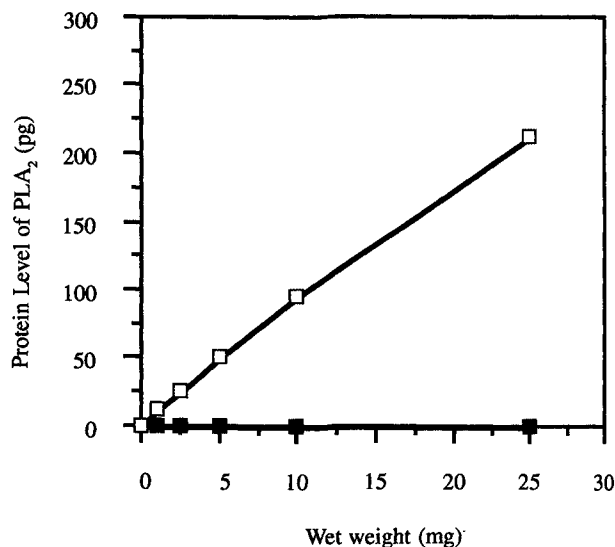


FIG. 1. Levels of group I- and II-phospholipase A₂ (PLA₂) as a function of wet weight of gingival tissue. Gingival tissue from a patient with periodontal disease was taken at the time of surgical therapy. The level of II-PLA₂ increased with the increase of tissue weight, but no I-PLA₂ was found.

activity.⁸ It has also been shown that II-PLA₂ activities found in inflamed sites other than those due to periodontitis, such as rheumatoid arthritis,⁴ Crohn's disease⁵ and septic shock,¹¹ are well correlated with disease activity, and thus II-PLA₂ is believed to play an important role in the initiation and propagation of the inflammatory process in these diseases. Recently, we showed that GCF sampled from sites of active periodontitis had higher PLA₂ activity than those from quiescent sites, although we could not identify whether the activity belonged to group I or II.⁸ These activities detected in GCF were thought to be those induced in the inflamed gingival tissues followed by secretion into the gingival crevice. In contrast, none of the samples tested had any detectable levels of I-

Table 1. The levels of I-PLA₂ and II-PLA₂

Site	PLA ₂ (pg/mg wet weight)	
	Group I	Group II
1	n.d.	12
2	n.d.	169
3	n.d.	120
4	n.d.	88
5	n.d.	5
6	n.d.	206
7	n.d.	216
8	n.d.	131
9	n.d.	6
10	n.d.	14
11	n.d.	87
12	n.d.	17
13	n.d.	17

Levels of I-PLA₂ and II-PLA₂ were determined by RIA. A wide range of II-PLA₂ levels was observed in gingival tissues, but no I-PLA₂ was found in any of the samples tested. n.d., not detectable.

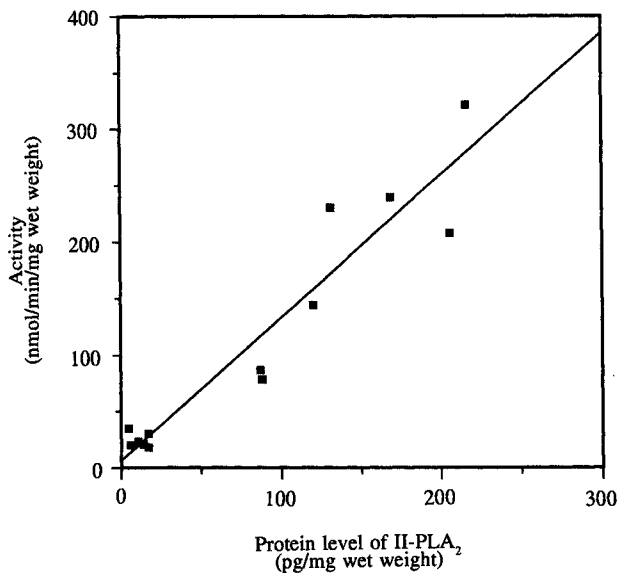


FIG. 2. Relationship between the activity and level of II-PLA₂. There is a linear correlation between II-PLA₂ activity and level ($r = 0.907$, Pearson product moment correlation coefficients).

PLA₂ using this assay system. These results suggest that the PLA₂ activity found in the GCF of patients with periodontal disease is not I-PLA₂ but II-PLA₂.

It has been reported that serum contains many factors that modulate the activity of PLA₂, for example, an activating protein such as PLA₂-activating protein¹² and an inhibitory protein such as C3dg.¹³ Up to now it has not been clarified whether the elevated PLA₂ activity in GCF and gingival tissue is due to an increase in enzyme concentration or to direct activation of the enzyme. Figure 2 shows the relationship between the level of II-PLA₂ determined by RIA and the activity determined by biochemical assay in the 13 samples. As can be seen, a highly significant correlation ($r = 0.907$, $p < 0.001$) between the level and activity of II-PLA₂ was observed. These findings indicate that the PLA₂ activity detected in GCF was attributable primarily to an increase in the protein concentration of II-PLA₂, probably via *de novo* protein synthesis in the gingiva, and not to activation of the enzyme. This close correlation between the level and activity of II-PLA₂ agrees well with previous reports on septic shock,¹¹ malaria,¹⁴ or Crohn's disease and ulcerative colitis.¹⁵

We showed that inflammatory cytokines such as IL-1 β and TNF- α induced, whereas an anti-inflammatory cytokine, TFG- β , inhibited the synthesis and secretion of II-PLA₂ in cultured rat gingival fibroblastic cells.^{6,7} It is well known that these

cytokines are present in GCF and levels of IL-1 β in gingival tissue correlate well with disease activity of periodontitis.¹⁶ Although it was not possible to identify the source of II-PLA₂ in gingival tissue from this study, it is considered that gingival cells in the inflamed sites are one of the most likely candidates.

In summary, the present results suggest that PLA₂ activity detected in GCF does not belong to I-PLA₂, but to II-PLA₂. It is also suggested that measurement of either the level of II-PLA₂ in biopsy samples of inflamed gingiva or the enzyme activity in GCF may be useful for detecting sites of active disease in periodontitis.

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