# PHOSPHOLIPASE $A_2$ ACTIVITY IN THE PLASMA MEMBRANE OF HUMAN POLYMORPHONUCLEAR LEUKOCYTES

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## 1. Introduction

The recognition that the plasma membrane (PM) is the site of reception, modulation and transfer of signals that lead to the cell's responses has aroused much recent interest in the possibility that modification of membrane phospholipids (PL) is important in *trans*-membrane signalling. Modification of PL requires the participation of phospholipases. For example, the removal of fatty acids by deacylating enzymes may trigger their replacement with fatty acids differing in physical properties, thereby changing membrane fluidity.

The polymorphonuclear leukocyte (PMN) provides an example of a cell thought to undergo PL modification as part of its typical functions, including chemotaxis and phagocytosis [1–3]. The pathways of PL synthesis and degradation have been well studied in PMN [4]. Phospholipase $A_2$ ($PLA_2$) and lysophospholipase activities have been identified in human and rabbit PMN [5–8]. The $PLA_2$ of PMN is particulate and is predominantly associated with the membranes of both specific and azurophil granules [5,7], but may also occur in other membranes including the PM [5,7].

It has been speculated that in PMN the arachidonic acid precursor for prostaglandin synthesis is preferentially hydrolyzed by PM $PLA_2$ from lecithin that in turn is derived from PM phosphatidylethanolamine via the methylation pathway [3]. However, evidence permitting the conclusion that these metabolic events, including the action of a $PLA_2$, actually occur in the PM, has not been presented.

We now demonstrate the presence of $PLA_2$ in the PM of human PMN, using a cell fractionation procedure that provides a highly purified PM preparation in high yield [9]. Thus, an essential enzyme in the metabolism of PL of PMN is present in a strategic location in or at the cell surface. This may support the contention that functionally important PL alterations occur in the PM.

## 2. Experimental

Human PMN isolation and fractionation were carried out as in [9]. Particulate PMN fractions were disrupted by aspiration through a 25 gauge needle and/or by freeze–thaw treatment before measurement of PMN activities and protein. These fractions were also extracted with 0.16 N $H_2SO_4$ to solubilize PLA and a bactericidal protein as in [10,11]. This was necessary to optimize the assay of PLA activity.

Bacteria: Rough *Escherichia coli* were cultured in a triethanolamine-buffered (pH 7.75–7.9) minimal salts medium as in [10]. Bacterial PL were labeled specifically in the 1- or 2-acyl position by growth of *E. coli* in medium containing [1-$^{14}$C]palmitic acid or [1-$^{14}$C]oleic acid (both 60 Ci/mol (Amersham Searle Corp.)) [12].

PLA activity of PMN fractions toward [$^{14}$C]fatty-acid-labeled *E. coli* (autoclaved to inactivate bacterial PLA and to render bacterial PL readily degradable by added PLA [5]) was measured during incubation at 37°C for 1 h in a mixture also containing 80 mM buffer (typically Tris–HCl (pH 7.5)) and 10 mM $CaCl_2$. Hydrolysis and its positional specificity were determined by thin-layer chromatographic separation of lipid-extracted free fatty acids, monoacylphosphatides and diacylphosphatides and counting of the labeled lipid species as in [8].

The presence of a granule-associated bactericidal/permeability-increasing protein (BPI) [10] in the vari-



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ous subcellular fractions was determined by measuring the bactericidal activity of these fractions towards rough *E. coli*. Essentially all bactericidal activity of crude human PMN fractions toward rough *E. coli* is attributable to BPI [10]. Incubations and measurements of bacterial viability were carried out as in [10]. The concentration of BPI in acid extracts was also measured immunochemically by the method in [13], using specific goat anti-human BPI serum.

Lysozyme activity in the various subcellular fractions was measured turbidometrically by the rate of lysis of *Micrococcus lysodeikticus* [14]. Protein was measured by the method in [15].

## 3. Results and discussion

PM and human PMN can be obtained in high yield and purity, using nitrogen cavitation to break the cells and differential centrifugation, as in [9].

Table 1 shows the distribution and recovery of protein, lysozyme, PLA activity, and a potent granule-associated bactericidal protein [10], among PMN fractions obtained by this method. This fractionation procedure produced the expected protein and lysozyme distribution, indicating that the PM containing preparation (pellet 3) is almost free of granule contents. This conclusion is further supported by the finding that only 1% of granule-associated BPI is found in pellet 3. Thus, BPI is a useful marker for human PMN granules, particularly because this protein is tightly associated with the granule membranes and is not released during fractionation [11].

The granule fraction also contains most of the recovered PLA activity. However, in contrast to the distribution of lysozyme and BPI activities, nearly 20% of the recovered PLA activity is present in pellet 3 (PM containing fraction). The PLA activity of pellet 3 is equally distributed between endoplasmic reticulum (ER) and PM vesicles (table 2). ER and PM vesicles were separated by dextran density gradient centrifugation [9]. The ER, a site of considerable PLA activity in other cells [16], is the subcellular fraction with the highest specific PLA activity in PMN. The remaining PLA activity in the purified PM fraction nevertheless exceeds what could be accounted for by a contamination with ER and/or granules. Moreover, the tight association of the PLA activity with any PMN-membrane preparation in which it occurs renders it unlikely that the phospholipase(s) of the PMN is(are) redistributed during fractionation.

Table 1
Subcellular distribution of phospholipase activity in human PMN

| Fraction | Protein | | Lysozyme | | BPI | | Phospholipase A | |
|---|---|---|---|---|---|---|---|---|
| | μg | Recovery(%)[a] | Units[b] | Recovery(%) | Units[c] | Recovery(%) | Units[d] | Recovery(%) |
| Whole cavitated sample | 704.5 ± 47 (4) | 100 | 180 (2) | 100 | 8.4 (2) | 100 | 25.8 ± 8.2 (7) | 100 |
| Pellet 1 (nuclei + intact cells) | 99 ± 19 (5) | 14 | 14 ± 8 (5) | 8 | 1.9 (2) | 22.6 | 1.7 ± 0.7 (7) | 7 |
| Pellet 2 (granules) | 110 ± 9 (5) | 15.6 | 168 ± 11 | 90 | 6.4 ± 1.1 (5) | 76.2 | 11.0 ± 2.6 (7) | 43 |
| Pellet 3 (plasma membrane-rich) | 7.9 ± 0.7 (5) | 1.1 | 6 ± 1 | 3.5 | 0.1 ± 0.1 (4) | 1.2 | 2.8 ± 0.9 (7) | 11 |
| Supernatant 3 | 295 ± 34 (5) | 41.8 | n.d. | | <0.4 (1) | – | <0.1 (1) | – |

[a] Recovery in each fraction is given as % of activity in whole cavitated sample: Activities are given as arbitrary units/$10^7$ PMN (mean ± SEM); (*n*) = number of independent determinations carried out at least in duplicate; n.d. = not determined
[b] Lysozyme, one unit = $\Delta A$ 450 × $10^3$/min; [c] BPI, one unit = 90% loss of viability of 5 × $10^6$ *E. coli*; [d] Phospholipase A, one unit = 1% hydrolysis of 5 nmol phospholipid/h

Nitrogen cavitation and differential centrifugation were carried out as in [9]. For detailed description of assays for phospholipase A, bactericidal (BPI) and lysozymal activities see references in section 2

Table 2[a]
Subfraction of pellet 3

| Fraction | Protein recovery(%) | Phospholipase A recovery(%) |
|---|---|---|
| Pellet 3 | 100 | 100 |
| Plasma membranes | 68.4 ± 18.2 (6) | 59 ± 9.4 (5) |
| Endoplasmic reticulum | 18.7 ± 13 (6) | 66 ± 13 (5) |

[a] See legend of table 1

The PLA activity of all subcellular fractions shows a high degree of positional specificity. Using *E. coli*, autoclaved after labeling with [$^{14}$C]oleic acid (>95% incorporated into the 2-position of *E. coli* phospholipids) or with [$^{14}$C]palmitic acid (>90% incorporated into the 1-position) [12], as substrate for assays of PLA activity, revealed that ≥80% of hydrolysis by all PMN fractions is attributable to $PLA_2$ activity, confirming observations on crude human PMN fractions [5,7]. The pH profiles of $PLA_2$ activity in all fractions are superimposable, showing a main peak between pH 7–8 and a second minor peak at pH 5.5 (not shown). At pH 5.5 as well as at pH 7.5 activity requires $Ca^{2+}$ and is abolished by 5 mM EDTA in line with our report on crude human PMN fractions [7].

Thus, although these studies suggest that more than one $PLA_2$ activity may be present in human PMN, differing in pH optimum, we have so far found no evidence of heterogeneity related to cellular (membrane) distribution. However, functional differences in $PLA_2$ activity may exist on the basis of cellular location rather than catalytic properties of the enzyme [5].

We have shown that PMN–$PLA_2$ is involved in degradation of PL of bacteria ingested and killed by the PMN [12,17,18]. Net degradation of PL of several Gram-negative bacterial species appears linked to the bactericidal action of BPI [8], which facilitates in highly specific manner hydrolysis by PMN–$PLA_2$ [17].

Since BPI is only found in association with the granules, it is tempting to suggest an anatomical segregation of $PLA_2$ function in this cell:

(1) Phospholipase(s) $A_2$ associated with the membranes of both specific and azurophil granules [5,7] that participate, after degranulation, in the intravacuolar antibacterial assault of the PMN on ingested bacteria;

(2) $PLA_2$ activity in other PMN membranes, presumably concerned with endogenous PL metabolism.

Our evidence of $PLA_2$ activity in the plasma membrane of the PMN should provide further impetus to the search for the connection between surface perturbation, activation of $PLA_2$, PL remodeling and conversion of released arachidonic acid to biologically active derivatives, and the triggering of specific PMN functions.

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