Brief report

Cytosolic phospholipase A\textsubscript{2} type IVA is present in human red cells

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Phospholipase A\textsubscript{2} type IVA (IVAPLA\textsubscript{2}) is a cytosolic enzyme that on activation selectively releases arachidonic acid (AA) from cell membrane phospholipids. Both AA and lysophospholipid, products of the enzyme reaction, can function as signal transducers in cellular interactions. The enzyme is present in most cells, including polymorphs, eosinophils, and platelets. This study used affinity purification to extract IVAPLA\textsubscript{2} from red cell lysate prepared from leukocyte- and platelet-depleted human blood to overcome the masking effect of hemoglobin on Western blot detection. We show that IVAPLA\textsubscript{2} is present in red cells as a 90-kDa protein. (Blood. 2004;103:3562-3564)

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Introduction

In their 1997 review Minetti and Low\textsuperscript{1} suggest that the cell signaling components of erythrocytes allow them to respond to their cellular environment and interact with other blood or endothelial cells. Phospholipase A\textsubscript{2} group IVA (IVAPLA\textsubscript{2}) is a widely distributed enzyme\textsuperscript{2} with cell signaling importance. It is upregulated by a rise in intracellular calcium, triggering phosphorylation and transfer of active enzyme from cytosol to membrane. On membrane binding it preferentially releases the fatty acid arachidonic acid (AA) from the sn2 position of the membrane phospholipid. Both enzymic products, AA and lysophospholipid, have second messenger properties,\textsuperscript{3} whereas AA is the source for the eicosanoids. A study\textsuperscript{4} of arachidonate metabolism in mammalian red cells concluded that the AA-derived 12-hydroxyeicosatetraenoic acid metabolite, promoted by Ca\textsuperscript{2+} ionophore stimulation, came from red cells and not from contaminating platelets and leukocytes.

IVAPLA\textsubscript{2} is activated by mitogen-activated protein (MAP) kinases,\textsuperscript{5,6} and the 2 MAP kinase isoforms extracellular signal regulated kinase 1 (ERK1) and ERK2 required for IVAPLA\textsubscript{2} phosphorylation are present in human red cells.\textsuperscript{7} IVAPLA\textsubscript{2} is present in neutrophils\textsuperscript{8} and platelets,\textsuperscript{9} and it has been measured in eosinophils.\textsuperscript{10} As the red cell and the leukocytes differentiate from the same common pluripotent stem cell, it is possible that the mature red cell may also contain this enzyme. IVAPLA\textsubscript{2} has not previously been observed in human red cells. Detection of IVAPLA\textsubscript{2} in the red cell is difficult, as substrate assays lack specificity for the group IVA enzyme and hemoglobin masks detection by Western blot. We have used an affinity purification technique prior to Western blotting to overcome this difficulty.

Phospholipase A\textsubscript{2} type IVA (IVAPLA\textsubscript{2}) is a cytosolic enzyme that on activation selectively releases arachidonic acid (AA) from cell membrane phospholipids. Both AA and lysophospholipid, products of the enzyme reaction, can function as signal transducers in cellular interactions. The enzyme is present in most cells, including polymorphs, eosinophils, and platelets. This study used affinity purification to extract IVAPLA\textsubscript{2} from red cell lysate prepared from leukocyte- and platelet-depleted human blood to overcome the masking effect of hemoglobin on Western blot detection. We show that IVAPLA\textsubscript{2} is present in red cells as a 90-kDa protein. (Blood. 2004;103:3562-3564)

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Study design

The human monocytic cell line U937 (American Type Culture Collection [ATCC] CRL2093) was cultured in roller bottles and harvested at 3.6 × 10\textsuperscript{10} cells. A human red cell lysate was prepared from 100-mL date-expired, leukocyte- and platelet-depleted red cell transfusion unit, frozen, and stored overnight at −80°C to lyse the cells. The IVAPLA\textsubscript{2} from both the U937 cytosol and the red cell lysate was separately affinity purified by chromatography on Prosep-G columns (Millipore, Bedford, MA) that had anti-IVAPLA\textsubscript{2} (Binding Site, Birmingham, United Kingdom) coupled to it. The columns were washed with 25 mM Tris (tris(hydroxymethyl)aminomethane) buffer, pH 7.4, containing 500 mM sodium chloride, and the bound IVAPLA\textsubscript{2} was eluted under denaturing conditions with 2% sodium dodecyl sulfate (SDS).

To assess the possible contribution of leukocytes and platelets to the IVAPLA\textsubscript{2} in the red cell transfusion unit, leukocyte- and platelet-rich preparations were examined. An expired transfusion pack of human platelets was washed with phosphate-buffered saline (PBS), resuspended in 10 mL PBS, and frozen at −80°C. Leukocytes were isolated by centrifuging at 200 g to remove platelets. The buffy coat was washed with water to remove the red cells, resuspended in 0.5 mL PBS, and frozen at −80°C. Table 1 shows the cell composition of the blood preparations.

The cell lysates were Western blotted after polyacrylamide gel electrophoresis in 4% to 12% gradient gels, blotted onto nitrocellulose membrane, and probed with a monoclonal anti-IVAPLA\textsubscript{2} (Santa Cruz Biotechnology, Santa Cruz, CA), then with peroxidase-conjugated rabbit antimmunoglobulin G (IgG; Dako, Glostrup, Denmark) (Figure 1).

The IVAPLA\textsubscript{2} present in the blood cell preparations and the affinity column eluates were measured by double dilution titers on Western blots by using IVAPLA\textsubscript{2} expressed as a glutathione-S-transferase (GST)–tagged

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The immunoblot (Figure 1) shows that the IV APLA2 present in U937 cells before and after affinity purification is a 90-kDa protein. The molecular weight of IV APLA2 on the basis of amino acid sequence is 85 kDa. Molecular weights reported for this protein have ranged from 110 kDa when purification is for U937 cells (lane 2) is effectively bound to the column, as no 90-kDa protein in Spodoptera frugiperda (Sf9) insect cells as a standard covering the range 0.62 to 5.0 μg IVAPLA2/mL; results are shown in Table 2.

**Results and discussion**

In the immunoblot the IV APLA2 from the 3 different sources migrated on polyacrylamide gel electrophoresis (PAGE) as a 90-kDa protein. The molecular weight of IVAPLA2 on the basis of amino acid sequence is 85 kDa. Molecular weights reported for this protein have ranged from 110 kDa when purified from U937 cells, 110 kDa when expressed and purified from Sf9 insect cells, 94 kDa in Chinese hamster ovary cells, and 90 kDa when purified from human platelets. Although our findings agree with the last report, there has never been an adequate explanation proposed for the differences in molecular weights reported for this protein.

In the blood used for affinity purification, leukocytes were reduced to less than 3% and platelets to less than 0.4% of that found in whole blood; nevertheless, it is possible that the remaining leukocytes and platelets could have contributed to the IV APLA2 isolated by the affinity purification from red cells. By using the results from the Western blot titering experiment we were able to estimate the IV APLA2 content of the nonerythroid cells (Table 2).

The U937 cell contained 292 fg IV APLA2/cell, consistent with overexpression of the protein; platelets contained 2.8 fg/cell. Because we could not detect IV APLA2 in the leukocyte, we assumed the amount present to be less than the detection limit of the titering experiment (ie, 0.62 μg/mL), and by using this figure we estimated the leukocyte has less than 7.8 fg/cell. However, the true figure is likely to be much less than this level. This figure now allows us to estimate the possible contribution of leukocytes and platelets to the IV APLA2 measured in the red cell affinity-purified material. In 100 mL red cells applied to the affinity column; platelets contributed 0.1 × 10^9 × 2.8 × 10^{-15} g = 0.28 × 10^{-6} g (ie, 0.28 μg). Leukocytes contributed 0.02 × 10^9 × 7.8 × 10^{-15} g = 0.16 × 10^{-6} g (ie, 0.16 μg). Total platelet and leukocyte contribution = 0.44 μg.

This amount is 1.5% of the 29.6 μg eluted from the red cell affinity column. We, therefore, conclude that the remaining platelets or leukocytes in the red cell transfusion pack only contributed a very small amount to the IV APLA2 found and that the detected IV APLA2 was derived from the red cells. The estimate of IVPLA2 in the red cells can now be calculated as 0.16 fg/cell and assumes an equivalent recovery from the affinity purification as for U937 cells. This estimate is of the same order as found in eosinophils by another technique (ie, 0.38 fg/cell).

In conclusion this report provides clear evidence that IV APLA2 is present in human red cells. Its function within the red cell is unclear, but it may provide a large mobile store for AA, allowing its release at sites in which there is a metabolic interaction between red cells and other cells, for example, platelets.

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References

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