Cytosolic phospholipase A2 type IVA is present in human red cells

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Phospholipase A2 type IVA (IVAPLA2) is a cytosolic enzyme that on activation selectively releases arachidonic acid (AA) from cell membrane phospholipids. Both AA and lysophospholipid, products of the enzymatic 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 IVAPLA2 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 IVAPLA2 is present in red cells as a 90-kDa protein.

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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 × 10¹⁰ cells, and a cell cytosol was prepared.11 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 IVAPLA2 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-IVAPLA2 (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 IVAPLA2 was eluted under denaturing conditions with 2% sodium dodecyl sulfate (SDS).

To assess the possible contribution of leukocytes and platelets to the IVAPLA2 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 from 60 mL EDTA (ethylenediaminetetraacetic acid) blood by centrifuging at 200g 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 on nitrocellulose membrane, and probed with a monoclonal anti-IVAPLA2 (Santa Cruz Biotechnology, Santa Cruz, CA), then with peroxidase-conjugated rabbit antirabbit immunoglobulin G (IgG; Dako, Glostrup, Denmark) (Figure 1).

The IVAPLA2 present in the blood cell preparations and the affinity column eluates were measured by double dilution titers on Western blots by using IVAPLA2 expressed as a glutathione-S-transferase (GST)–tagged...
protein in Spodoptera frugiperda (Sf9) insect cells11 as a standard covering the range 0.62 to 5.0 μg IVAPL₂/mL; results are shown in Table 2.

### Results and discussion

The immunoblot (Figure 1) shows that the IVAPL₂ present in U937 cells (lane 2) is effectively bound to the column, as no IVAPL₂ was detected in the eluate (lane 3). SDS elution from the column showed the presence of a 90-kDa protein (lane 4) which was also present in the affinity-purified red cells (lane 5) and in the unpurified platelets (lane 7). Column recovery of IVAPL₂ from the U937 lysate was 29%. Identification of the IVAPL₂ depends on 2 antibodies. The affinity purification antibody is against the last 24 amino acids of the molecule’s C terminus and has no homology with any other protein, whereas the probing antibody is against the first 216 amino acids at the N terminus. Figure 1 provides clear evidence that IVAPL₂ present in U937 cells before and after affinity purification is also detected in red cells and platelets. Failure to detect IVAPL₂ in the leukocyte preparation (lane 8) is probably a result of insufficient numbers of IVAPL₂-containing leukocytes in the preparation (Table 1).

In the immunoblot the IVAPL₂ from the 3 different sources migrated on polyacrylamide gel electrophoresis (PAGE) as a 90-kDa protein. The molecular weight of IVAPL₂ on the basis of amino acid sequence is 85 kDa.12 Molecular weights reported for this protein have ranged from 110 kDa when purified from U937 cells,13 100 kDa when expressed and purified from Sf9 insect cells,11 94 kDa in Chinese hamster ovary cells,14 and 90 kDa when purified from human platelets.15 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 IVAPL₂ isolated by the affinity purification from red cells. By using the results from the Western blot titering experiment we were able to estimate the IVAPL₂ content of the nonerythroid cells (Table 2).

The U937 cell contained 292 fg IVAPL₂/cell, consistent with overexpression of the protein; platelets contained 2.8 fg/cell. Because we could not detect IVAPL₂ 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 IVAPL₂ measured in the red cell affinity-purified material. In 100 mL red cells applied to the affinity column; platelets contributed 0.1 × 10⁹ × 2.8 × 10⁻¹⁵ g = 0.28 × 10⁻¹⁶ g (ie, 0.28 μg). Leukocytes contributed 0.02 × 10⁹ × 7.8 × 10⁻¹⁵ g = 0.16 × 10⁻¹⁶ g (ie, 0.16 μg). Total platelet and leukocyte contribution was 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 IVAPL₂ found and that the detected IVAPL₂ was derived from the red cells. The estimate of IVAPL₂ 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).10

In conclusion this report provides clear evidence that IVAPL₂ 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.16

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References

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