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Purification and Characterization of a Cytosolic, 42 kDa and Ca^{2+}-dependent Phospholipase A_2 from Bovine Red Blood Cells : Its Involvement in Ca^{2+}-dependent Release of Arachidonic Acid from Mammalian Red Blood Cells-

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It has become evident that a Ca^{2+}-dependent release of arachidonic acid (AA) and subsequent formation of bioactive lipid mediators such as prostaglandins and leukotrienes in red blood cells (RBCs) can modify physiological functions of neighboring RBCs and platelets. Here we identified a novel type of cytosolic PLA_2 in bovine and human RBCs and purified it to apparent homogeneity with a 14,000-fold purification. The purified enzyme, termed $rPLA_2$, had a molecular mass of 42 kDa and revealed biochemical properties similar to group IV $cPLA_2$, but showed different profiles from $cPLA_2$ in several column chromatographies. Moreover, $rPLA_2$ did not react with any of anti-$cPLA_2$ and anti-$sPLA_2$ antibodies and was identified as an unknown protein in matrix-assisted laser desorption/ionization time-of-flight mass spectrometric analysis. Divalent metal ions tested exhibited similar effects between $rPLA_2$ and $cPLA_2$, whereas mercurials inhibited $cPLA_2$ but had no effect on $rPLA_2$. Antibody against the 42 kDa protein not only precipitated the $rPLA_2$ activity, but also reacted with the 42 kDa protein from bovine and human RBCs in immunoblot analysis. The 42 kDa protein band was selectively detected in murine fetal liver cells known as a type of progenitor cells of RBCs. It was found that EA4, a derivative of quinone newly developed as an inhibitor for $rPLA_2$, inhibited a Ca^{2+} ionophore-induced AA release from human and bovine RBCs, indicating that this enzyme is responsible for the Ca^{2+}-dependent AA release from mammalian RBCs. Finally, erythroid progenitor cell assay utilizing diaminobenzidine staining of hemoglobinized fetal liver cells showed that $rPLA_2$ detectable in erythroid cells was down-regulated when differentiated to non-erythroid cells. Together, our results suggest that the 42 kDa $rPLA_2$ identified as a novel form of Ca^{2+}-dependent PLA_2 may play an important role in hemostasis, thrombosis and/or erythropoiesis through the Ca^{2+}-dependent release of AA.