Stimulation of platelets with platelet-activating factor induces changes in the subcellular distribution and activity of the tyrosine kinase pp60

Christine T. Murphy and John Westwick

Department of Pharmacology, University of Bath, Claverton Down, Bath, Avon BA2 7AY

Activation of platelets by different agonists induces rapid tyrosine phosphorylation of a number of proteins by stimulating one or more protein tyrosine kinase (PTKase) [1]. The presence of several members of the src family of PTKases, namely pp60
, pp60
, pp61
, pp62
 and pp54/56
, has now been identified in platelets [2]. The major PTKase expressed in platelets is pp60
 which comprises 0.2-0.4% of total cell protein [3]. The abundance of pp60
 in platelets together with the increase in tyrosine phosphorylation of proteins upon platelet activation implicates pp60
 as having a role in the signal transduction mechanism(s) operating within these cells. The effect of PAF-stimulation on the number of proteins phosphorylated on tyrosine residues, together with the subcellular distribution of these phosphoproteins has been determined. Moreover, both the subcellular distribution and the in vitro kinase activity of the major PTKase, pp60
, has been monitored in platelets treated with PAF. The association of pp60
 and tyrosine phosphorylated protein(s) with phosphatidylinositol (PI)-kinase activity was also investigated. Washed rabbit platelets were prepared as previously described [4]. Immunoblotting with a specific monoclonal anti-phosphotyrosine antibody (PY20) detected four tyrosine phosphorylated proteins (52 - 62 kDa) in unstimulated platelets. Within 5s of stimulation with 300 nM PAF, phosphorylation of two groups of proteins in the molecular mass groups of 35 - 45 kDa and 66 - 90 kDa was detected. At 30s post-PAF a third group of proteins (90 - 150 kDa) was detected. These findings are in agreement with the temporal waves of phosphorylation previously detected in thrombin-stimulated platelets [1]. Cytoskeletal fractions, detergent soluble fractions and membrane skeletal fractions were prepared from equal numbers of platelets as previously described [5]. PAF-stimulation induced a rapid increase in the number of tyrosine phosphorylated proteins in both the cytoskeletal and the detergent soluble fraction. Immunoblotting was also used to determine the subcellular distribution of pp60
. In resting platelets pp60
 was located in the detergent soluble fraction and the membrane skeletal fraction, however, upon cell stimulation pp60
 became associated with the cytoskeletal fraction (Fig 1).

In order to investigate the difference in activity of pp60
 before and after stimulation with PAF, platelets were lysed and extracted in RIPA buffer, pp60
 was immunoprecipitated and its in vitro kinase activity was determined by autophosphorylation. There was a rapid decrease in the in vitro kinase activity of pp60
 immunoprecipitated from platelets which had been stimulated with PAF for 5 s compared to basal levels. After a 5 min stimulation with PAF the in vitro kinase activity of pp60
 was back to basal levels.

Abbreviations used: PTKase, protein tyrosine kinase; PAF, platelet activating factor; PI, phosphatidylinositol

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Cleveland analysis of autophosphorylated[32P]PP60
 using V8 protease [6] demonstrated that the phosphorylation of each of the peptides produced by protease cleavage, paralleled total pp60
 phosphorylation. Tyrosine phosphorylated proteins and pp60
 were found to become rapidly associated with and to co-precipitate with PI-kinase activity after platelet stimulation with PAF (300 nM).

The findings from this study demonstrate that upon activation with PAF, there is a rapid translocation of pp60
 from the detergent soluble fraction to the cytoskeletal platelet fraction. This rapid translocation is coincident with a decrease in the in vitro kinase activity of pp60
 immunoprecipitated from PAF-stimulated platelets. PP60
 was found to become rapidly associated with PI-kinase activity upon platelet stimulation with PAF, indicating the rapid formation of a signalling complex upon PAF-receptor ligation.

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