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PROPERTIES OF PHOSPHATIDYLINOSITOL KINASE IN HUMAN PLATELETS.
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We have reported the specific ³²P-labelling in phosphatidyl-
inositol-4-monophosphate (PIP) of intact platelets upon addition
of the agents which elevate intracellular cAMP (Thrombos. Res. 44,
155, 1986). This event may be catalyzed by the action of PI-kinase,
the properties of which has not been elucidated yet. Thereby,
attempts were made to assay and to characterize PI-kinase of
human platelets. Fresh lysed platelets prelabelled with ³²P in
cold Tris-HCl buffer containing 2mM EGTA were incubated at 37 C
in the presence of MgCl₂ for designated times and the phospho-
lipids were extracted and analyzed by thin layer chromatography.
³²P-labelling in PIP was gradually increased in consort with the
decreased labelling in PI-4,5-bisphosphate. As the changes in the
labelling was not affected by the presence of apyrase and as the
radioactive inositol trisphosphate was not detected, it was
suggested that the changes is due to the action of phosphomono-
esterase rather than PI-kinase or phospholipase C. When ³²P-ATP
was added to non-labelled lysed platelets upon incubation, ³²P was
labelled only into PIP and the amount was markedly increased
until 5min. after incubation. Since the labelling was strongly
inhibited by apyrase, it likely reflects the activity of PI-kinase.
The activity of PI-kinase thus measured required Mg²⁺ strictly
for the activity and the maximal activity was obtained in the
presence of 30mM Mg²⁺. In contrast, it was markedly inhibited in
the presence of Ca²⁺ (as low as 2mM Ca²⁺ in the presence of 2mM
EGTA), which was compatible with our previous findings with intact
platelets. The activity of A-kinase was not inhibited by a low
concentration of Ca²⁺. Furthermore, the activity was inhibited by
cAMP or dbcAMP in a dose related manner and no enhancement of the
activity was obtained by the addition of catalytic subunit of
A-kinase, though a significant reduction in the activity was
observed in the presence of inhibitor protein to A-kinase. From
these observations, the following conclusions were obtained; 1) The
activity of PI-kinase in lysed platelets may be determined by
pulse labelling with ³²P-ATP. 2) It requires Mg²⁺ absolutely and
is inhibited by a very low concentration of Ca²⁺. 3) PI-kinase is
activated by A-kinase but the activated enzyme is inhibited by
cAMP, suggesting the presence of feedback mechanism.

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PLATELET-FIBRIN CLOTS FORMED BY THROMBIN SELECTIVELY RETAIN
PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE (PIP₂). John D. Vickers,
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It is established that stimulation of human platelets with
thrombin for 60 s in the absence of fibrinogen increases the
amount of PIP₂ compared with unstimulated controls (4.7 ± 0.24
nmol/10⁹ plat. vs 3.83 ± 0.14 nmol/10⁹ plat., p<0.01, n=8). How-
ever, stimulation with thrombin for 60 s in the presence of
fibrinogen causes a large decrease in the amount of PIP₂ that
can be extracted with acidified chloroform/methanol compared
with unstimulated controls (1.62 ± 0.39 nmol/10⁹ plat. vs 3.84 ±
0.44 nmol/10⁹ plat., p<0.001, n=6). Stimulation of rabbit plate-
lets with thrombin in the presence of fibrinogen also decreases
the amount of extractable PIP₂ (60% at 60 s, p<0.001, n=8).
Similar decreases in amount can not be demonstrated for phospha-
tidylinositol 4-phosphate, phosphatidylinositol, phosphatidic
acid or phosphatidylcholine under the same conditions, indicat-
ing that the decrease is specific for PIP₂. With rabbit plate-
lets, polymerized fibrin formed by reptilase, which does not
stimulate platelets or induce clot retraction, does not cause
the decrease in extractable PIP₂ (3.06 ± 0.05 nmol/10⁹ plat.
were extracted compared with 3.18 ± 0.07 nmol/10⁹ plat. without
reptilase). However, stimulation of rabbit platelets with ADP in
the presence of polymerizing fibrin formed by reptilase causes a
larger decrease in extractable PIP₂ (to 2.54 ± 0.19 nmol/10⁹
plat., p<0.05, n=4) than is caused by ADP and fibrinogen alone
(to 2.87 ± 0.06 nmol/10⁹ plat., p<0.05, n=4). Inhibition by
glycyl-L-prolyl-L-arginyl-L-proline of polymerization of fibrin
formed by the action of thrombin prevents the large decrease in
the amount of extractable PIP₂ (4.37 ± 0.30 nmol/10⁹ plat. were
extracted) from human platelets. These results indicate that the
interaction of polymerizing fibrin with stimulated platelets is
required for the decrease in PIP₂. The decrease in extractable
PIP₂ seen with polymerizing fibrin can not be explained by in-
creased degradation of PIP₂ to IP₃ or PIP. Thus, when human or
rabbit platelets are stimulated with thrombin in the presence of
fibrinogen, an association of polymerizing fibrin with the stimu-
lated platelets occurs that leads to decreased extractability
of PIP₂. This may mean that PIP₂ forms a specific association
with platelet proteins that are involved in clot retraction.

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TURNOVER OF THE PHOSPHOMONOESTER GROUPS OF POLYPHOSPHO-
INOSITIDES IN UNSTIMULATED HUMAN PLATELETS.
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The metabolic activity of the polyphosphoinositides in
unstimulated human platelets was studied by 1) short-term
labelling with ³²P-P₁, 2) by replacement of ³²P-
P₁ from pre-labelled platelets with unlabelled phosphate
and 3) by depriving the cells of metabolic ATP. Under
short-term labelling conditions, the 4- and 5-phosphates of
phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol
4,5-bisphosphate (PIP₂) had the same specific radioactivity
as the γ-phosphate of metabolic ATP. The specific radioactivity
of the 1-phosphates of phosphatidylinositol, PIP and PIP₂,
was similar, but only 4-13 % as compared to the γ-phosphate of
ATP. When ³²P-P₁ pre-labelled platelets were incubated
with up to 25 mM of unlabelled phosphate, the displacement of
the ³²P-label from PIP, PIP₂ and metabolic ATP followed
similar kinetics. Inhibition of ATP regeneration in
³²P-P₁ pre-labelled platelets resulted in a rapid fall
in ³²P-metabolic ATP with much slower fall in ³²P-PIP₂.
³²P-PIP increased initially and decreased thereafter in
parallel with PIP₂. However, ATP turnover was not
abolished, as indicated by the marked (25 % of the control)
incorporation of extracellular ³²P-P₁ into PIP and
PIP₂ in metabolically inhibited platelets. This low
phosphate turnover may explain the relative resistance of PIP
and PIP₂ to metabolic inhibition.
We conclude that PIP and PIP₂ are present as a single
metabolic pool in human platelets. Turnover of the 4- and
5-phosphates of PIP and PIP₂ in unstimulated platelets is
as rapid as that of the γ-phosphate of metabolic ATP, and
accounts for about 7 % of basal ATP consumption.

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EVIDENCE THAT ABNORMAL PLATELET AGGREGATION IN SPONTANEOUSLY
HYPERTENSIVE RATS IS LINKED WITH PHOSPHOINOSITIDES TURNOVER AND
PHOSPHORYLATION OF 47,000 DALTON PROTEIN. Huzoor-Akbar and
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We have shown earlier that abnormal platelet aggregation in
spontaneously hypertensive rats (SHR) is not caused by
prostaglandins (Thromb. Res. 41, 555-566, 1986). In this study
platelets from SHR and normotensive (Wistar Kyoto, WKY) rats
were used to examine the role of phosphoinositides (PIs) and
protein phosphorylation in increased platelet activation in
hypertension. Thrombin (0.05 U/ml) induced rapid hydrolysis of
phosphatidylinositol-4,5-bis-phosphate (PIP₂), phosphatidyl-
inositol-4-phosphate (PIP), and phosphatidylinositol (PI) in
(³²P)-PO₄ labeled platelets. However, significantly greater
hydrolysis of PIP₂ and PI was seen in SHR platelets than in WKY
platelets (see Table). Thrombin also caused two- to three-fold
increased accumulation of phosphatidic acid (PA) in SHR
platelets than in WKY platelets (see Table).

	% CHANGE IN	TIME (SECONDS)				
		3	5	15	30	240
PIP ₂	WKY	-15±3	-13±5	-15±4	-4±5	+7±7
	SHR	-23±4	-32±2	-25±4	-8±5	+15±7
PIP	WKY	-14±3	-8±3	-4±6	+3±4	+7±4
	SHR	-18±3	+7±5	+5±3	+20±4	+22±5
PI	WKY	-4±4	-13±4	-8±4	-1±3	+9±5
	SHR	-7±4	-12±3	-24±7	-5±4	+0±4
PA	WKY		+8±2	+28±12	+42±6	+106±35
	SHR		+19±10	+89±9	+134±25	+219±39

Thrombin caused phosphorylation of 18,000 Dalton (P18) and 47,000
Dalton (P47) proteins in SHR and WKY Platelets. Significantly
increased phosphorylation of P47 was seen at 5, 15, 60 and 240
seconds of incubation with thrombin in SHR platelets (60%, 68%,
98% and 91%) than in WKY platelets (13%, 37%, 44% and 47%). The
extent of P18 phosphorylation was same in both SHR and WKY
platelets. Aspirin (500 μM) did not affect phosphorylation of
P47 or P18 in SHR or WKY Platelets. These data lead us to
suggest that increased turnover of PIs and increased
phosphorylation of P47 are involved in abnormal platelet
aggregation in SHR (Supported in part by the COHC grant #86-01-A
and the Ohio University College of Osteopathic Medicine).