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Changes in the platelet phosphoinositides during the first minute after stimulation of washed rabbit platelets with thrombin

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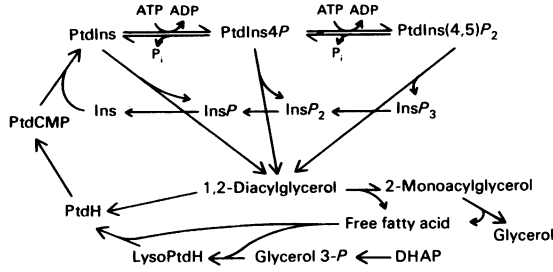
Experiments with washed platelets from rabbits demonstrate that stimulation with a low concentration of thrombin (0.1 unit/ml) that causes maximal aggregation and partial release of granule contents does not significantly decrease the amount of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] at 10s; this contrasts with ADP stimulation. The amount of PtdIns(4,5)P_2 was significantly decreased by a higher concentration of thrombin (0.3 unit/ml). Increased turnover of the PtdIns(4,5)P_2 at 60s was indicated by changes in labelling with [^{3}H]glycerol in platelets stimulated with both concentrations of thrombin. An unexpected observation with the lower thrombin concentration was a significant increase in the amount of phosphatidylinositol (PtdIns) at 10s. This contrasts with data from other laboratories, which indicate that thrombin causes a significant decrease in PtdIns. At 60s, with the lower concentration of thrombin, PtdIns was significantly decreased. With the higher concentration of thrombin there was a significant decrease in the amount of PtdIns at 10s, in keeping with the data from other laboratories. The initial increase in PtdIns may not have been observed by other investigators because higher concentrations of thrombin were used. The reaction involved in this initial increase in the amount of PtdIns does not appear to be increased degradation of PtdIns4*P* or PtdIns(4,5)P_2, since their total amount was unchanged at 10s. The magnitude of the increase in PtdIns is such that more than the existing pool of phosphatidic acid would have to be converted into PtdIns to account for the increase. It is suggested that synthesis of phosphatidic acid *de novo* from dihydroxyacetone phosphate and glycerol 3-phosphate might be the source of phosphatidic acid, which leads to increased PtdIns at 10s with the lower concentration of thrombin. Thus it appears that the initial response of platelets to thrombin does not require an early change in PtdIns(4,5)P_2 and may involve stimulation of synthesis *de novo* of PtdIns via phosphatidic acid.

Degradation of the phosphoinositides by phospholipase C to 1,2-diacylglycerol and phosphorylation of the 1,2-diacylglycerol to phosphatidic acid (Scheme 1) has been proposed to be a primary event in the initial response of platelets to thrombin (Rittenhouse, 1982). After stimulation with ADP, thrombin or collagen, one of the earliest changes in platelets labelled with [^{32}P]phosphate is an increase in the ^{32}P labelling of phosphatidic acid (Lloyd *et al.*, 1972; Lloyd & Mustard, 1974). Lapetina & Cuatrecasas (1979) have shown more recently that an increase in the ^{3}H labelling of phosphatidic acid in platelets prelabelled with [^{3}H]arachidonate precedes any other reported change in the lipids of thrombin-stimulated platelets. By adding a high concentration of thrombin (17 units/ml) to 10^{10} platelets/ml, Rittenhouse-Simmons (1979) showed an early increase in the amount of 1,2-diacylglycerol, the intermediate between PtdIns and phosphatidic acid. Activation of this pathway in thrombin-stimulated platelets has been correlated with the freeing of arachidonic acid (Bell *et al.*, 1979; Lapetina & Cuatrecasas, 1979; Rittenhouse-Simmons, 1979; Bell & Majerus, 1980; Broekman *et al.*, 1980; Chau & Tai, 1981). However, the action of phospholipase A_2

Abbreviations used: PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate; PtdIns4*P*, phosphatidylinositol 4-phosphate; PtdIns, phosphatidylinositol; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.



Scheme 1. *Pathways of phosphoinositide metabolism, including polyphosphoinositide interconversion, phosphoinositide turnover and the pathway of synthesis de novo*

Abbreviations used only in the Figure: PtdCMP, phosphatidylcytidine monophosphate; PtdH, phosphatidic acid; Ins, inositol; Ins*P*, InsP_2, InsP_3, inositol mono-, bis- and tris-phosphate; DHAP, dihydroxyacetone phosphate.

(Bills *et al.*, 1976; Rittenhouse-Simmons *et al.*, 1977; McKean *et al.*, 1981; Billah *et al.*, 1981) or acyltransferases (Irvine & Dawson, 1979; Irvine, 1982) could also be involved in the freeing of arachidonic acid.

Degradation of PtdIns(4,5)P_2 rather than PtdIns to 1,2-diacylglycerol, with the freeing of inositol phosphates, has been proposed to be involved in stimulus–response coupling in a variety of tissues (Michell, 1982; Hawthorne, 1982; Putney, 1982; Berridge, 1982), although the relationship of the decrease to Ca^{2+} mobilization is still unclear (Michell, 1982; Putney, 1982; Hawthorne, 1982).

We have reported that PtdIns(4,5)P_2 decreases when platelets are stimulated with ADP (Vickers *et al.*, 1982), and Billah & Lapetina (1982) have reported that stimulation of horse platelets with 2 units of thrombin/ml causes a decrease in PtdIns(4,5)P_2. Although Bell & Majerus (1980) concluded that the amounts of PtdIns(4,5)P_2 and PtdIns4*P* were not changed in thrombin-stimulated human platelets, the data presented in their paper showed larger changes than we observed in ADP-stimulated rabbit platelets (Vickers *et al.*, 1982) or Billah & Lapetina (1982) observed in thrombin-stimulated horse platelets. More recently, Agranoff *et al.* (1983) have shown a small decrease in the labelling of PtdIns(4,5)P_2 and an increase in inositol 1,4,5-trisphosphate within 5s of stimulation of human platelets with 5 units of thrombin/ml. This result indicates that PtdIns(4,5)P_2 is degraded by phospholipase C.

In the experiments reported in the present study, we have examined changes in the amount and labelling with [^{32}P]phosphate and [^{3}H]glycerol of the phosphoinositides of rabbit platelets suspended in a Tyrode/albumin solution in which they are responsive to all aggregating agents. We examined (*a*) whether there are detectable changes in PtdIns(4,5)P_2 in response to stimulation of the platelets with thrombin at a time when ADP is known to cause a decrease in the amount of PtdIns(4,5)P_2 and (*b*) the relationship to aggregation and the release reaction of the changes in PtdIns and phosphatidic acid that have been previously reported to occur in thrombin-stimulated platelets. Determination of both labelling and chemical amounts permits a distinction to be made between changes in phospholipids owing to a change in their rate of synthesis, which would alter the amount of the phospholipid, and a change in the rates of both synthesis and degradation (turnover), which may or may not alter the amount, but would change the specific radioactivity.

Materials and methods

Thrombin (bovine) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Phospholipid standards were from Serdary Research Laboratories (London, Ont., Canada), except for PtdIns(4,5)P_2 and PtdIns4*P*, which were from Sigma. Silica-gel H60 precoated thin-layer plates (Merck) were from Brinkmann Instruments (Rexdale, Ont., Canada). Solvents for t.l.c. were distilled before use.

[^{3}H]Glycerol (10Ci/mmol), 5-hydroxy-[2-^{14}C]-tryptamine-creatinine sulphate (60mCi/mmol) and carrier-free [^{32}P]P_i were purchased from New England Nuclear (Dorval, PQ, Canada).

Preparation of prelabelled washed rabbit platelets

Suspensions of washed rabbit platelets were prepared by the method of Ardlie *et al.* (1971). The usual first washing solution (Ca^{2+}-free Tyrode solution) was replaced with a Ca^{2+}- and phosphate-free Tyrode solution supplemented with 5mM-Hepes. Platelets were prelabelled in the first washing solution by incubation at 37°C for 1h with carrier-free [^{32}P]P_i (20μCi/ml) and [^{3}H]glycerol (20μCi/ml), and the unincorporated label was removed by centrifuging and resuspending the

platelets twice in Ca^{2+}-free Tyrode solution as described previously (Vickers *et al.*, 1982). Platelets were finally suspended in Tyrode solution with 0.35% albumin and apyrase at a concentration capable of converting 0.25 μmol of ATP into AMP in 120s at 37°C. The platelet count was adjusted to 10^9 platelets/ml.

Under these labelling conditions, the phosphoinositides and phosphatidic acid showed rapid labelling while the [^{32}P]P_i was present, and a slow decrease in labelling after the [^{32}P]P_i was removed and the platelets were resuspended in a medium containing unlabelled phosphate. These changes have been studied up to 3h after removal of the labelled precursor, the period in which the experiments in this study were done. During this period the average specific radioactivity of the phosphate groups in the phosphoinositides and phosphatidic acid decreased in parallel with the specific radioactivity of ATP. Thus a steady state had apparently been established between the label in the phosphate groups of the ATP, phosphatidic acid and phosphoinositides.

When the platelets were labelled with [^{3}H]glycerol, washed and resuspended in a medium containing no glycerol, it appeared that the ^{3}H labelling of the phosphoinositides and phosphatidic acid was also in a steady state. It is not possible, on the basis of these experiments, to account for differences in the specific radioactivities of the phospholipids that could exist, because there are pools of the inositol phospholipids and phosphatidic acid which do not turn over in the unstimulated platelets.

To examine the release of granule contents from platelets stimulated with thrombin, platelets were prepared as described above, except that they were labelled with 5-hydroxy[^{14}C]tryptamine for 15min at the end of the 1h incubation in the first washing solution (Packham *et al.*, 1977). Release of serotonin was determined by rapidly transferring the platelet sample from the aggregometer cuvette to a centrifuge tube and centrifuging at 8000*g* for 1.5min; radioactivity was then determined in a sample of the supernatant fluid.

Thrombin stimulation of prelabelled platelets

Platelets prelabelled with [^{32}P]P_i and [^{3}H]glycerol were treated with thrombin at 0.1 or 0.3unit/ml. Aggregation was measured in a Payton aggregation module (Payton Associates, Scarborough, Ont., Canada). The reaction was terminated at specified times by the addition of chloroform/methanol extraction solvent directly to the cuvette. The lipids were extracted with a neutral solvent and then with an acidic solvent as previously described (Vickers *et al.*, 1982).

Fractionation of platelet lipids and determination of amount of radioactivity

Platelet phospholipid extracts were fractionated on two-dimensional thin-layer chromatograms, and the locations of the phospholipids were identified by autoradiography and by viewing under u.v. light after spraying with Rhodamine 6G. The silica gel containing the phospholipids was scraped into test tubes for digestion with $HClO_4$ before phosphate analysis (Chalvardjian & Rudnicki, 1970; Duck-Chong, 1979) and determination of radioactivity. The methods have been described in detail previously (Vickers *et al.*, 1982).

Data analysis

Since the experimental samples were paired and the individual data were not normally distributed, the statistical significance was determined by using a paired *t* test on logarithmically transformed values.

Results

Two concentrations of thrombin were used in these experiments. Thrombin at a concentration of 0.1unit/ml caused rapid shape change and maximal aggregation, with 33.1 ± 0.7 and 53.9 ± 1.2% release of 5-hydroxy[^{14}C]tryptamine from prelabelled platelets at 10s and 60s respectively. With 0.3unit of thrombin/ml, release was 67.6 ± 0.7 and 80 ± 1.4% at 10 and 60s respectively.

Changes in the phosphoinositides in response to a low concentration of thrombin

Stimulation of platelets with the lower concentration of thrombin (0.1unit/ml) had no effect on the amount of PtdIns(4,5)P_2 or its labelling with [^{32}P]P_i or [^{3}H]glycerol at 10s (Table 1). However, by 60s, the [^{3}H]glycerol specific radioactivity of PtdIns(4,5)P_2 was significantly decreased, although the amount and ^{32}P labelling were unchanged.

In contrast, PtdIns4*P* showed significant increases in labelling with both [^{3}H]glycerol and [^{32}P]P_i at 10s and significant increases in amount and labelling with both radioisotopes at 60s (Table 2).

Changes in PtdIns were dependent on the time of examination (Table 3). At 10s the amount of PtdIns was significantly increased. By 60s the amount of PtdIns was significantly less than in the controls, the ^{32}P specific radioactivity was increased and the ^{3}H labelling was decreased.

Consistent with previous reports (Lloyd & Mustard, 1974), the amount of phosphatidic acid and its ^{32}P labelling and specific radioactivity were increased significantly, except for the amount at 10s (Table 4). In contrast, no change was seen in

Table 1. *Changes in the amount, labelling and specific radioactivity of PtdIns(4,5)P_2 in platelets prelabelled with [^{32}P]P_i and [^3H]glycerol on stimulation with 0.1 unit of thrombin/ml*

Results are the means ± S.E.M. of data from seven experiments. Abbreviation: NS, not significant.

Parameter	Time (s)	Control	Treated	*n*	Significance
Amount					
(pmol/10^6 platelets)	10	2.58±0.13	2.59±0.14	12	NS
	60	2.65±0.12	2.71±0.11	13	NS
[^{32}P]P_i					
Labelling (d.p.m./10^6 platelets)	10	34.9±2.22	34.2±2.15	13	NS
	60	36.4±2.04	35.5±2.13	13	NS
Sp. radioactivity (d.p.m./pmol)	10	14.2±1.23	13.9±1.34	12	NS
	60	14.1±1.15	13.4±1.07	13	NS
[^{3}H]Glycerol					
Labelling (d.p.m./10^6 platelets)	10	8.49±0.54	8.38±0.43	13	NS
	60	9.06±0.46	8.34±0.36	13	NS
Sp. radioactivity (d.p.m./pmol)	10	3.36±0.22	3.30±0.20	12	NS
	60	3.46±0.18	3.14±0.18	13	$P<0.05$

Table 2. *Changes in the amount, labelling and specific radioactivity of PtdIns4P in platelets prelabelled with [^{32}P]P_i and [^3H]glycerol on stimulation with 0.1 unit of thrombin/ml*

Results are the means ± S.E.M. of data from seven experiments. Abbreviation: NS, not significant.

Parameter	Time (s)	Control	Treated	*n*	Significance
Amount					
(pmol/10^6 platelets)	10	2.40±0.21	2.41±0.22	12	NS
	60	2.31±0.16	2.93±0.15	13	$P<0.005$
[^{32}P]P_i					
Labelling (d.p.m./10^6 platelets)	10	10.0±0.61	10.5±0.55	13	$P<0.05$
	60	9.87±0.50	17.5±0.93	13	$P<0.001$
Sp. radioactivity (d.p.m./pmol)	10	4.35±0.42	4.66±0.57	12	NS
	60	4.60±0.50	6.03±0.33	13	$P<0.001$
[^{3}H]Glycerol					
Labelling (d.p.m./10^6 platelets)	10	5.02±0.26	5.46±0.26	13	$P<0.05$
	60	5.26±0.22	7.78±0.47	13	$P<0.001$
Sp. radioactivity (d.p.m./pmol)	10	2.20±0.14	2.39±0.16	12	NS
	60	2.38±0.16	2.67±0.13	13	NS

Table 3. *Changes in the amount, labelling and specific radioactivity of PtdIns in platelets prelabelled with [^{32}P]P_i and [^3H]glycerol on stimulation with 0.1 unit of thrombin/ml*

Results are the means ± S.E.M. of data from seven experiments. Abbreviation: NS, not significant.

Parameter	Time (s)	Control	Treated	*n*	Significance
Amount					
(pmol/10^6 platelets)	10	12.55±0.93	14.69±0.74	12	$P<0.01$
	60	14.15±0.81	12.12±0.71	13	$P<0.05$
[^{32}P]P_i					
Labelling (d.p.m./10^6 platelets)	10	13.3±1.21	14.2±1.32	12	NS
	60	14.3±1.18	14.4±1.18	13	NS
Sp. radioactivity (d.p.m./pmol)	10	1.01±0.13	0.98±0.07	12	NS
	60	1.05±0.11	1.22±0.11	13	$P<0.05$
[^{3}H]Glycerol					
Labelling (d.p.m./10^6 platelets)	10	41.8±2.94	44.2±2.26	12	NS
	60	44.3±2.21	35.6±2.19	13	$P<0.005$
Sp. radioactivity (d.p.m./pmol)	10	3.13±0.30	3.05±0.12	12	NS
	60	3.18±0.12	3.05±0.29	13	NS

Table 4. *Changes in the amount, labelling and specific radioactivity of phosphatidic acid in platelets prelabelled with [^{32}P]P_i and [^{3}H]glycerol on stimulation with 0.1 unit of thrombin/ml*

Results are the means ± S.E.M. of data from seven experiments. Abbreviation: NS, not significant.

Parameter	Time (s)	Control	Treated	*n*	Significance
Amount					
(pmol/10^6 platelets)	10	1.62±0.17	1.78±0.15	13	NS
	60	1.74±0.19	2.87±0.19	12	$P<0.001$
[^{32}P]Phosphate					
Labelling (d.p.m./10^6 platelets)	10	0.49±0.03	1.27±0.10	12	$P<0.001$
	60	0.48±0.04	9.56±0.40	13	$P<0.001$
Sp. radioactivity (d.p.m./pmol)	10	0.37±0.09	0.72±0.07	12	$P<0.001$
	60	0.35±0.08	3.57±0.32	12	$P<0.001$
[^{3}H]Glycerol					
Labelling (d.p.m./10^6 platelets)	10	2.70±0.37	2.78±0.24	12	NS
	60	2.15±0.17	8.84±0.58	13	$P<0.001$
Sp. radioactivity (d.p.m./pmol)	10	1.79±0.30	1.59±0.15	12	NS
	60	1.43±0.20	3.14±0.18	12	$P<0.001$

Table 5. *Percentage changes in the amount, labelling and specific radioactivity of PtdIns(4,5)P_2, PtdIns4P, PtdIns and phosphatidic acid in platelets prelabelled with [^{32}P]P_i and [^{3}H]glycerol and stimulated with 0.3 unit of thrombin/ml, compared with controls*

Paired samples of platelets (10^9 platelets/ml) were stirred with either 0.3 unit of thrombin/ml or Tyrode solution for 10 or 60s. The reaction was terminated by addition of chloroform/methanol. The lipids were extracted, fractionated, and the amount and radioactivity were determined as described in the Materials and methods section. The results with the thrombin-treated samples are expressed as a percentage of the results with samples to which Tyrode solution had been added. Data are the means of duplicate values from five experiments: $*P<0.05$, $**P<0.01$, $***P<0.001$.

			[^{32}P]Phosphate		[^{3}H]Glycerol	
Lipid	Time (s)	Amount	Labelling	Sp. radioactivity	Labelling	Sp. radioactivity
PtdIns(4,5)P_2	10	92.9**	88.1***	95.6**	90.9*	98.5
	60	103.8	108.1*	103.7*	88.7	87.5*
PtdIns4*P*	10	97.4	99.3	100.1	105.1	107.7
	60	159.1***	187.4***	115.3*	151.8***	95.7
PtdIns	10	91.8**	81.9	88.3	95.7	104.8
	60	80.4**	90.1	112.8*	73.0***	92.5***
Phosphatidic acid	10	112.5	393***	238**	98.2	55.7
	60	303.1***	2688***	831***	592***	186***

^{3}H labelling or specific radioactivity of phosphatidic acid at 10s, although by 60s both were significantly increased.

Changes in phosphoinositides in response to a high concentration of thrombin

PtdIns(4,5)P_2 showed significant decreases in amount, in ^{32}P labelling and specific radioactivity and in [^{3}H]glycerol labelling at 10s (Table 5) in response to the higher concentrations of thrombin (0.3unit/ml). At 60s there was a significant increase in ^{32}P labelling and a significant decrease in [^{3}H]glycerol specific radioactivity of PtdIns(4,5)P_2. In contrast, the changes in PtdIns4*P* were similar to those in response to the lower concentration of thrombin.

In response to the higher concentration of thrombin, PtdIns did not show the initial increase in the amount seen with the low concentration of thrombin at 10s (Table 5); it decreased significantly in amount at both 10 and 60s. The absence of a significant change in labelling with [^{32}P]P_i at 60s, when the specific radioactivity had increased and the ^{3}H labelling and specific radioactivity had decreased significantly, was similar to the results with the lower concentration of thrombin.

Like the changes in PtdIns4*P*, the changes in phosphatidic acid were similar to those caused by

the low concentration of thrombin (Table 5), but were larger.

Discussion

The observation that the amount and labelling of PtdIns(4,5)P_2 were unchanged by the lower concentration of thrombin (0.1 unit/ml) that caused platelet aggregation and about 50% release of amine-storage-granule contents at 60s indicates that a decrease in PtdIns(4,5)P_2 is not necessary for the initial platelet response. However, when a stronger thrombin stimulus (0.3 unit/ml) was used, there was a significant decrease in the amount of PtdIns(4,5)P_2 at 10s. The data in the studies of the ^{32}P and [^{3}H]glycerol labelling of PtdIns(4,5)P_2 suggest that, with the lower concentration of thrombin, increased turnover of the PtdIns(4,5)P_2, which could have occurred without a change in amount, did not occur during the first 10s of the platelet response. The significant decrease in the [^{3}H]glycerol specific radioactivity of PtdIns(4,5)P_2 at 60s in platelets stimulated with both concentrations of thrombin is compatible with increased turnover of PtdIns(4,5)P_2 at this time. For the higher concentration of thrombin, the labelling data are compatible with increased degradation of PtdIns(4,5)P_2 at 10s.

There are several possible explanations for the difference between our data and those of Billah & Lapetina (1982) and Agranoff *et al.* (1983), who found a decrease in PtdIns(4,5)P_2 on stimulation with thrombin. As indicated in the introduction, higher concentrations of thrombin (2 or 10 units/ml) were used in their studies, which undoubtedly led to more rapid changes. In addition, the platelets were from different species: horse (Billah & Lapetina, 1982), human (Agranoff *et al.*, 1983) and rabbit (the present study), and different suspending media were used.

In platelets stimulated with 0.1 unit of thrombin/ml, the amount of PtdIns was significantly increased at 10s. This contrasts with the observations by other investigators, which were obtained with higher concentrations of thrombin (Broekman *et al.*, 1980; Lapetina *et al.*, 1981). Again, the effects observed are dependent on the concentration of thrombin used, and with 0.3 unit of thrombin/ml in the present study the amount of PtdIns was decreased. Using a high concentration of thrombin (10 units/ml), Agranoff *et al.* (1983) found a small increase in the ^{32}P labelling of PtdIns at 10s, which was not statistically significant. Since the amounts of PtdIns(4,5)P_2 and PtdIns4*P* were not decreased, it is unlikely that the increase in PtdIns with 0.1 unit of thrombin/ml was due to increased degradation or decreased synthesis of the polyphosphoinositides. The increase in ^{32}P specific radioactivity of phosphatidic acid is consistent with increased turnover of the phosphatidic acid being involved in the increased synthesis of the PtdIns. Since the ^{3}H specific radioactivity of the phosphatidic acid did not increase, it is unlikely that the source of the phosphatidic acid is the phosphoinositides or phosphatidylcholine and phosphatidylethanolamine, which have higher specific radioactivities, and did not decrease in amount. The most likely pathway leading to the increase in amount of PtdIns seems to be synthesis *de novo* from dihydroxyacetone phosphate and glycerol 3-phosphate. The presence of this pathway has been demonstrated in platelet membranes (Okuma *et al.*, 1973). Since, as discussed in the Materials and methods section, the specific radioactivity of phosphatidic acid decreases slowly in resting platelets, presumably owing to incorporation of labelled glycerol from degraded phospholipids and unlabelled dihydroxyacetone phosphate, the small decrease in phosphatidic acid ^{3}H specific radioactivity is consistent with acceleration of synthesis *de novo*. The observation that the ^{3}H specific radioactivity of phosphatidic acid is also decreased at 10s in platelets stimulated with 0.3 unit/ml is consistent with activation of synthesis *de novo* in platelets stimulated with 0.3 unit of thrombin/ml. The increase in PtdIns in this case may have preceded the 10s sampling.

The significant increases in amount and ^{3}H specific radioactivity of phosphatidic acid by 60s are consistent with increased synthesis of phosphatidic acid from 1,2-diacylglycerol resulting from degradation of the phosphoinositides. The decrease in PtdIns could be due to either degradation by a phospholipase C specific for PtdIns, which has been identified in platelets (Rittenhouse-Simmons, 1979; Billah *et al.*, 1979), and/or phosphorylation to PtdIns4*P* and PtdIns(4,5)P_2 to replace PtdIns(4,5)P_2 degraded by phospholipase C, as suggested by Agranoff *et al.* (1983).

The cause of the changes in PtdIns4*P* is not clear, although they are generally consistent with its position as an intermediate between PtdIns and PtdIns(4,5)P_2. It is likely that the early increase in PtdIns contributes to an increase in the synthesis of PtdIns4*P* observed at 60s, and that the increased turnover involved in the resynthesis of PtdIns(4,5)P_2 contributes to the changes in PtdIns4*P* labelling.

The results from these studies agree with published observations that thrombin stimulation of platelets can cause a decrease in PtdIns(4,5)P_2 and PtdIns and can increase phosphatidic acid in association with platelet aggregation and release of amine-storage-granule contents. When a lower concentration of thrombin is used, however, these studies indicate that synthesis *de novo* of phospha-

tidic acid and PtdIns is initiated before increased degradation of PtdIns(4,5)P_2 or PtdIns is detectable. It remains to be established whether the initial increase in phosphatidic acid is, in part or entirely, a product of synthesis *de novo*.

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