Biochem. J. (1987) 246, 109–114 (Printed in Great Britain)

Formation and metabolism of inositol 1,4,5-trisphosphate in human platelets

James L. DANIEL, Carol A. DANGELMAIER and J. Bryan SMITH
Temple University, Pharmacology Department and Thrombosis Research Center, 3400 N. Broad Street, Philadelphia, PA 19140, U.S.A.

1. *myo*-[^{3}H]Inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3], when added to lysed platelets, was rapidly converted into [^{3}H]inositol 1,3,4,5-tetrakisphosphate [Ins(1,3,4,5)P_4], which was in turn converted into [^{3}H]inositol 1,3,4-trisphosphate [Ins(1,3,4)P_3]. This result demonstrates that platelets have the same metabolic pathways for interconversion of inositol polyphosphates that are found in other cells. 2. Labelling of platelets with [^{32}P]P_i, followed by h.p.l.c., was used to measure thrombin-induced changes in the three inositol polyphosphates. Interfering compounds were removed by a combination of enzymic and non-enzymic techniques. 3. Ins(1,4,5)P_3 was formed rapidly, and reached a maximum at about 4 s. It was also rapidly degraded, and was no longer detectable after 30–60 s. 4. Formation of Ins(1,3,4,5)P_4 was almost as rapid as that of Ins(1,4,5)P_3, and it remained detectable for a longer time. 5. Ins(1,3,4)P_3 was formed after an initial lag, and this isomer reached its maximum, which was 10-fold higher than that of Ins(1,4,5)P_3, at 30 s. 6. Comparison of the intracellular Ca^{2+} concentration as measured with fura-2 indicates that agents other than Ins(1,4,5)P_3 are responsible for the sustained maintenance of a high concentration of intracellular Ca^{2+}. It is proposed that either Ins(1,3,4)P_3 or Ins(1,3,4,5)P_4 may also be Ca^{2+}-mobilizing agents.

INTRODUCTION

In human platelets, as well as in many other cells, an early response to agonist stimulation is a rapid increase in metabolism of phosphoinositides. The primary event seems to be phosphodiesteratic cleavage of PtdIns(4,5)P_2 by a specific phospholipase C (Michell *et al.*, 1981). This reaction results in the production of two second messengers, diacylglycerol, an activator of protein kinase C (Nishizuka, 1984), and Ins(1,4,5)P_3, a compound which has been shown to liberate Ca^{2+} from internal storage sites (Streb *et al.*, 1983; Berridge & Irvine, 1984).

Further evidence has indicated that the metabolism of inositol phosphates is more complicated than was previously assumed. In addition to the expected Ins(1,4,5)P_3 isomer, many stimulated cells also show increases in Ins(1,3,4)P_3 (Irvine *et al.*, 1984, 1985; Burgess *et al.*, 1985). Batty *et al.* (1985) found Ins(1,3,4,5)P_4 in carbachol-stimulated rat brain slices. Downes *et al.* (1986) showed that the source of Ins(1,3,4)P_3 was not PtdIns(3,4)P_2, since this phospholipid could not be detected in rat parotid-gland slices, and they suggested that Ins(1,3,4,5)P_4 was the precursor of Ins(1,3,4)P_3. They have demonstrated that an enzyme from erythrocyte membranes can catalyse this conversion. Subsequently, formation of both Ins(1,3,4,5)P_4 and Ins(1,3,4)P_3 from [^{3}H]Ins(1,4,5)P_3 has been demonstrated in several different types of permeabilized or homogenized cells (Hawkins *et al.*, 1986; Hansen *et al.*, 1986; Rossier *et al.*, 1986; Stewart *et al.*, 1986). In addition, temporal studies indicate that Ins(1,4,5)P_3 is formed before Ins(1,3,4,5)P_4, which is subsequently converted into Ins(1,3,4)P_3 (Hawkins *et al.*, 1986; Hansen *et al.*, 1986).

Human platelets respond to a variety of stimuli and rapidly convert PtdIns(4,5)P_2 into diacylglycerol and InsP_3 (Watson *et al.*, 1984, 1985; Rittenhouse & Sasson, 1985; Daniel *et al.*, 1986). However, perhaps because of their limited capacity for uptake of [^{3}H]inositol, little is known about the formation of InsP_3 isomers and InsP_4 by stimulated platelets. By labelling polyphosphoinositides with [^{32}P]P_i, we have developed a method to measure changes in inositol polyphosphates. We have found that the metabolism of Ins(1,4,5)P_3 is similar to that of other cells and that the predominant isomer of InsP_3 in thrombin-stimulated human platelets is Ins(1,3,4)P_3.

MATERIALS AND METHODS

Materials

IP_3, 2,3-bisphosphoglycerate phosphatase (rabbit muscle) and inorganic pyrophosphatase (baker's yeast) were obtained from Sigma Chemical Co. [^{3}H]Ins(1,4,5)P_3 was from New England Nuclear, and [^{32}P]P_i was obtained from ICN. Fura-2 was obtained from Molecular Probes. Bovine thrombin was from Armour Pharmaceutical. Prepacked 25 cm Partisil 10 SAX columns were obtained from Whatman. Water and methanol were h.p.l.c.-grade.

Preparation of platelets

Human blood was taken by venipuncture from informed healthy volunteers into acid/citrate/dextrose (Aster & Jandl, 1964). Platelet-rich plasma obtained by centrifugation at 180 *g* for 15 min at ambient temperature was re-centrifuged (800 *g* for 15 min, ambient temperature). The platelet pellet was resuspended in 0.2 vol. of autologous platelet-poor plasma and incubated with [^{32}P]P_i (0.25 mCi/ml) for 1 h at 37 °C. In some

Abbreviations used: PtdIns(4,5)P_2, phosphatidylinositol 4,5-phosphate; InsP_3, inositol trisphosphate (isomeric form unspecified); Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; Ins(1,3,4)P_3, inositol 1,3,4-trisphosphate; Ins(1,3,4,5)P_4, inositol 1,3,4,5-tetrakisphosphate.

experiments fura-2 acetomethoxy ester (5 μM) was added after 30 min and the incubation continued for another 30 min. The platelets were separated from unincorporated [^{32}P]P_i by gel filtration on Sepharose 2B (Pharmacia) by using a Ca^{2+}-free Tyrode's buffer containing 0.2% albumin, 5 mM-glucose and 5 mM-Hepes (pH 7.3). The cell suspension was adjusted to a final concentration of 1×10^9 cells/ml.

Preparation of platelet lysate, and measurement of [^{3}H]Ins(1,4,5)P_3 metabolism

Platelet-rich plasma was centrifuged at 1000 *g* for 25 min. The platelet pellet was resuspended and adjusted to a concentration of 1×10^{10} cells/ml in a buffer consisting of 140 mM-potassium glutamate, 10 mM-EDTA, 17 mM-$MgCl_2$ and 10 mM-Hepes (pH 7.2). The cells were lysed by a single cycle of freezing and thawing.

In order to measure the conversion of [^{3}H]Ins(1,4,5)P_3 into other metabolites, 0.2 μCi of [^{3}H]Ins(1,4,5)P_3 and 20 μM unlabelled InsP_3 were added to 1 ml of platelet lysate in a buffer containing 10 mM-EDTA, 17 mM-$MgCl_2$, 5 mM-ATP, 5 mM-pyrophosphate and 2 mM-$CaCl_2$. The final Ca^{2+} concentration in this buffer was calculated to be approx. 10 μM. Samples (100 μl) were removed at various times and added to an equal volume of 1.2 M-$HClO_4$; they were then neutralized and analysed by h.p.l.c.

Activation of platelets

Activation of platelets (2.5 ml) was performed at 37 °C with stirring at 100 rev./min with a final concentration of 5 units of bovine thrombin/ml. Reactions were stopped by addition of an equal volume of ice-cold 1.2 M-$HClO_4$. Experiments with fura-2 were conducted in a similar fashion in water-jacketed cuvette in a Perkin–Elmer LS-5 spectrofluorimeter. Reactions were stopped by addition of 1 ml of ice-cold 2.1 M-$HClO_4$.

Measurement of Ca^{2+} with fura-2

Fura-2 fluorescence was monitored continuously by using settings of 340 nm (excitation) and 510 nm (emission). Fura-2 fluorescence signals were calibrated by the method of Pollack *et al.* (1986). $F_{max.}$ was determined by lysing the cells with 50 μM-digitonin in the presence of 1 mM-$CaCl_2$. $F_{min.}$ was determined by the addition of 10 mM-EGTA and 20 mM-Tris base. Correction was made for the presence of external dye.

Preparation of $HClO_4$ extracts for h.p.l.c.

Before further processing, 0.02 μCi of [^{3}H]Ins(1,4,5)P_3 was added to each sample. Samples were adjusted to pH 7.5–8.0 by addition of 2 M-K_2CO_3 and treated at 37 °C for 15 min with 50 μl of a solution of inorganic pyrophosphatase (50 units/ml) in 5 mM-$MgCl_2$/1.0 M-Tris/HCl (pH 8.0)/ml. This reaction was stopped by the addition of 0.1 vol. of 6.6 M-$HClO_4$. P_i was precipitated by addition of 267 μl of 0.02 M-ammonium molybdate/ml and 67 μl of 0.1 M-triethylamine/HCl (pH 5.0)/ml (Sugino & Miyoshi, 1964). The precipitate was removed by centrifugation (5000 *g* for 5 min). The supernatant was adjusted to pH 6–7 with 2 M-K_2CO_3 and treated with 200 mg of charcoal (Darco, type G-60) to remove nucleotides (Meek, 1986). Samples were left for 5 min on ice, and the charcoal was removed by centrifugation (5000 *g*). The clear supernatant was diluted 5-fold with water and passed over an NH_2-bonded phase column (type LC-NH2; Supelco, Bellefonte, PA, U.S.A.). After washing with 5 ml of water, the column was eluted with 500 μl of 1.5 M-NH_3 and the eluate freeze-dried in a Savant Speed-Vac Concentrator. Samples were dissolved in water for separation by h.p.l.c.

Separation of inositol phosphates by h.p.l.c.

Inositol phosphates were separated and analysed by a modification of the method of Irvine *et al.* (1985). The chromatography apparatus consisted of Waters U6K injector, two Waters M-6000A pumps, a model-660 gradient programmer, a pre-column filter (0.5 μm), a guard column packed with Whatman pre-column gel and a 0.46 cm × 25 cm Whatman Partisil 10 SAX separating column. The column was eluted with a flow rate of 1.5 ml/min and a 60 min linear gradient of water, as initial buffer, and 1.5 M-ammonium formate adjusted to pH 3.7 with H_3PO_4, as final buffer. A mixture of ATP, ADP, AMP and adenosine was included in all samples. The elution of the adenine-containing compounds was monitored with a Kratos Spectroflow variable-wavelength absorbance detector at 259 nm. Radioactivity was monitored with a Ramona-D (IN/US Service Corp., Fairfield, NJ, U.S.A.) flow detector equipped with a flow cell for [^{32}P]P_i. Fractions were also collected and analysed for both ^{3}H and ^{32}P in a Beckman LS 1800 liquid-scintillation counter.

RESULTS

H.p.l.c. of ^{32}P-labelled metabolites from control and stimulated platelets

Platelets labelled with [^{32}P]P_i show high incorporation into PtdIns(4,5)P_2, the precursor of Ins(1,4,5)P_3. However, the major problem with this approach is that many water-soluble metabolites in addition to inositol phosphates become labelled with [^{32}P]P_i. Major interference would come from nucleoside triphosphates, which are eluted in the same region of the h.p.l.c. gradient as for the inositol trisphosphates. Nucleotides were removed by the method of Meek (1986), who showed that one type of charcoal could totally remove interfering nucleotides with little or no loss of other phosphorylated compounds. A possible conflict with 2,3-bisphosphoglycerate was also anticipated, since we had previously shown that this compound can co-electrophorese with InsP_3 (Dangelmaier *et al.*, 1986).

The elution profile of the ^{32}P-labelled compounds from a neutralized $HClO_4$ extract of ^{32}P-labelled platelets is shown in Fig. 1. A profile obtained from a radioactivity detector equipped with a flow cell is shown, since this method of detection results in greater resolution than collection of fractions for scintillation counting. The profile from unstimulated cells (trace A) shows several major peaks. We have identified the major metabolites present in the labelled peaks. The peak labelled 'P_i' was found to co-migrate with P_i on two-dimensional paper chromatography (Holmsen *et al.*, 1983). Most of the radioactivity in this peak was removed by a method which specifically precipitates P_i (Sugino & Miyoshi, 1964). Most of the radioactivity in peak labelled 'FDP' in control samples was found to co-migrate with fructose bisphosphate on two-dimensional paper chromatography. The radioactivity in peak labelled 'ATP' may represent residual [^{32}P]ATP, since it was eluted in the

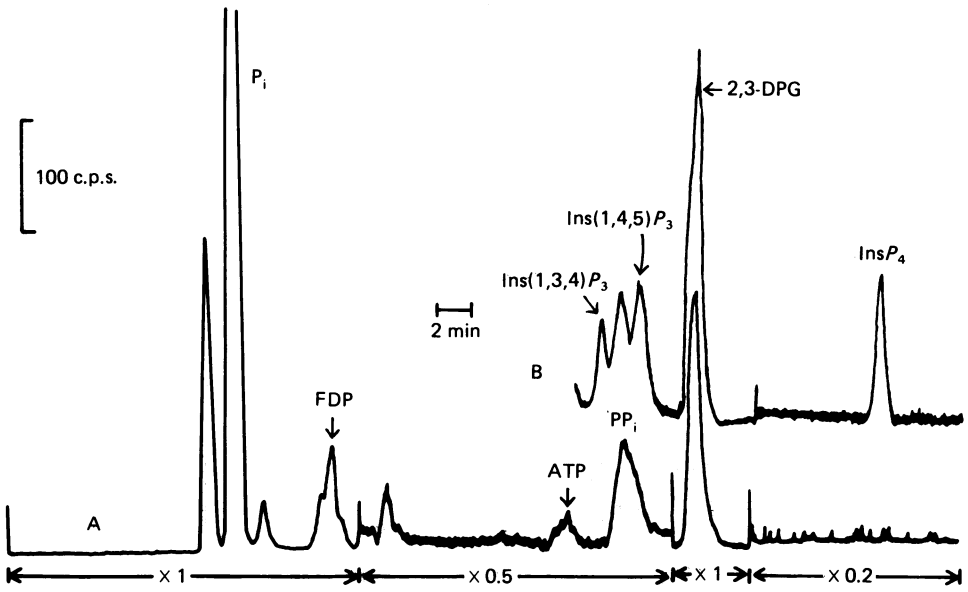

Fig. 1. H.p.l.c. radioactivity profiles of aqueous ^{32}P-labelled extracts prepared from control and thrombin-stimulated human platelets

Human platelets were incubated with [^{32}P]P$_i$, and neutralized $HClO_4$ extracts made from control cells (trace A) and cells incubated with thrombin (5 units/ml; trace B) for 4 s as described in the Materials and methods section. Nucleotides were removed by charcoal treatment. Samples were injected and h.p.l.c. was performed as described in the Materials and methods section. The traces were obtained from a Ramona-D radioactivity detector. The labelled peaks are described in the text.

same position as the ATP standard. The peak labelled 'PP$_i$' was identified as PP$_i$, since it was specifically removed by inorganic pyrophosphatase. The peak labelled '2,3-DPG' co-migrated with 2,3-bisphosphoglycerate and was specifically removed by treatment with 2,3-bisphosphoglycerate phosphatase. Stimulation with thrombin for 4 s results in the appearance of three new peaks. On the basis of their elution positions, we initially identified them as Ins(1,3,4)P_3, Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4.

Metabolism of [^{3}H]Ins(1,4,5)P_3 in platelet lysates, and identification of the three peaks formed in thrombin-stimulated platelets

The experiment illustrated in Fig. 1 suggested that platelets not only produced Ins(1,4,5)P_3 upon stimulation with thrombin, but also contained enzymes to convert this compound into Ins(1,3,4,5)P_4 and Ins(1,3,4)P_3. In order to confirm the latter, commercially available [^{3}H]Ins(1,4,5)P_3 was added to a lysate of human platelets, and conversion of Ins(1,4,5)P_3 into other metabolites was monitored by h.p.l.c. The rate of metabolism was controlled by the amount of unlabelled InsP_3 added and by including 5 mM-sodium pyrophosphate, an inhibitor of 5'-monoesterase (Hansen *et al.*, 1986). Metabolites were identified by their elution relative to the adenine nucleotides and to each other as described by Irvine *et al.* (1985) and Hansen *et al.* (1986). Fig. 2 shows that the first two metabolites formed are inositol bisphosphate and Ins(1,3,4,5)P_4. The Ins(1,3,4,5)P_4 is gradually converted into Ins(1,3,4)P_3 and the bisphosphate is more slowly converted into inositol monophosphate. This experiment indicates that platelets contain the kinase required to convert Ins(1,4,5)P_3 into Ins(1,3,4,5)P_4, and the latter can be converted into Ins(1,3,4)P_3.

To substantiate the identity of the ^{32}P-labelled

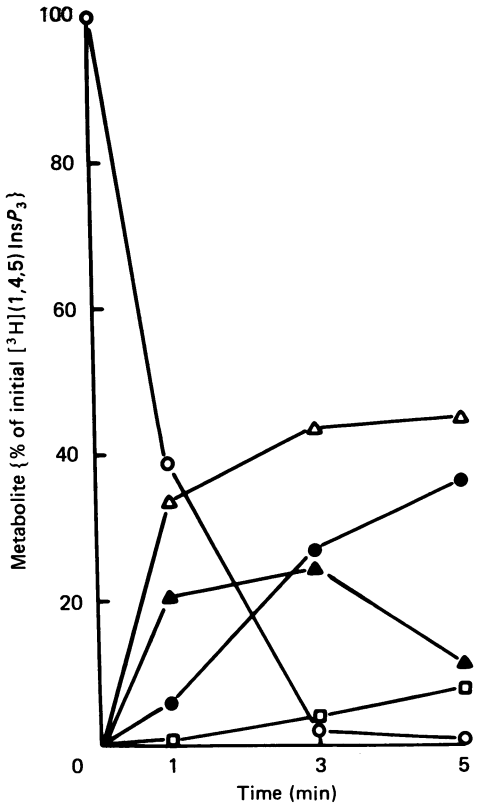

Fig. 2. Metabolic interconversions of [^{3}H]Ins(1,4,5)P_3 in a platelet lysate

This experiment was conducted as described in the Materials and methods section. Samples were neutralized, but did not undergo any other treatment before injection into the h.p.l.c. system. ○, Ins(1,4,5)P_3; ▲, Ins(1,3,4,5)P_4; ●, Ins(1,3,4)P_3; △, inositol bisphosphate; □, inositol monophosphate.

metabolites of human platelets that increase upon stimulation with thrombin, a neutralized $HClO_4$ extract of ^{32}P-labelled platelets that had been treated with thrombin for 4 s was mixed with both [^{3}H]Ins(1,4,5)P_3 and an extract from a platelet lysate that had been incubated with [^{3}H]Ins(1,4,5)P_3 for 5 min (see Fig. 2). Before mixing, the ^{32}P-labelled extract was treated to remove P_i and PP_i. As shown in Fig. 3, an exact correspondence was obtained between the elution of the ^{3}H-labelled metabolite and its corresponding ^{32}P-labelled metabolite. In addition, both [^{32}P]Ins(1,3,4)P_3 and [^{32}P]Ins(1,4,5)P_3 were found to co-migrate with an InsP_3 standard on two-dimensional paper chromatography.

Time course of interconversion of inositol polyphosphates

The kinetics of the formation of Ins(1,4,5)P_3 and its conversion into Ins(1,3,4,5)P_4 and Ins(1,3,4)P_3 after maximal stimulation of ^{32}P-labelled human platelets with thrombin is shown in Fig. 4(*a*), which is representative of three other similar experiments. Half-maximal concentrations of both Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 were reached in less than 2 s. Ins(1,4,5,)P_3 was rapidly degraded, and concentrations were not significantly above basal after 1 min. In contrast, measurable concentrations of Ins(1,3,4,5)P_4 were detected at both 5 and 10 min after stimulation. Formation of Ins(1,3,4)P_3 lagged behind the other two, and reached a peak at 1 min after stimulation, consistent with the idea it is a product derived from Ins(1,3,4,5)P_4. The peak value of Ins(1,3,4)P_3 was over 10-fold greater than that of either of its precursors, and it was maintained at high levels over the 10 min time course of the experiment.

Our measurements of intracellular Ca^{2+} concentrations with fura-2 have demonstrated that platelet stimulation by thrombin normally resulted in large prolonged

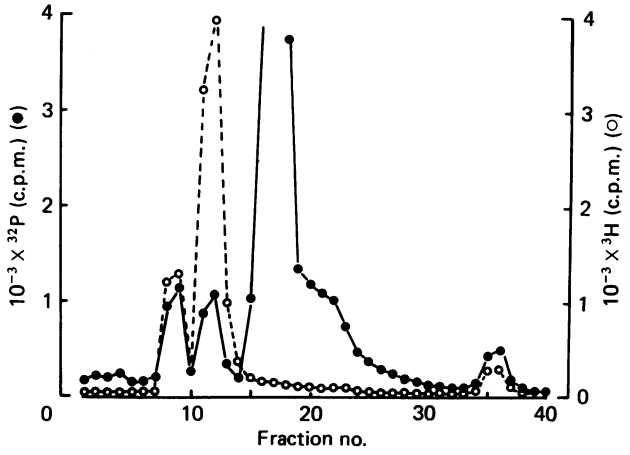

Fig. 3. Co-elution of inositol [^{32}P]polyphosphates with [^{3}H]inositol polyphosphates

A neutralized $HClO_4$ extract from ^{32}P-labelled platelets that had been stimulated with thrombin for 4 s was treated as described in the Materials and methods section. Another extract was prepared by incubating [^{3}H]Ins(1,4,5)P_3 with platelet lysate for 5 min as in Fig. 2. The two extracts were mixed with 0.02 μCi of [^{3}H]Ins(1,4,5)P_3 and injected into the h.p.l.c. Samples were collected and analysed for the presence of ^{3}H (○) and ^{32}P (●) by liquid-scintillation spectrometry.

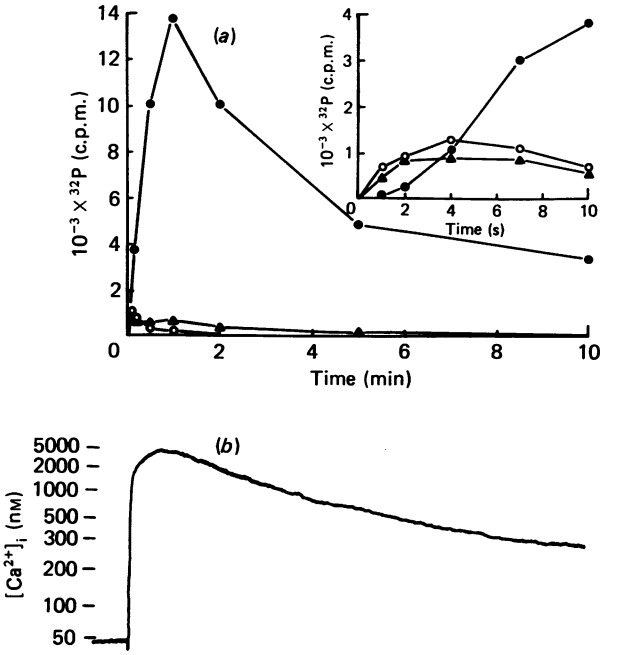

Fig. 4. Time course of the formation of inositol polyphosphates and of cytosolic Ca^{2+} mobilization

Thrombin (5 units/ml) was added to ^{32}P-labelled platelets in the fluorimeter. (*a*) At indicated times the reaction was stopped by addition of $HClO_4$, and the amount of Ins(1,4,5)P_3 (○), Ins(1,3,4,5)P_4 (▲) and Ins(1,3,4,)P_3 (●) was determined as outlined in the Materials and methods section. The inset shows the first 10 s of the time course. (*b*) Recording of the fluorescence of fura-2 (calibrated as $[Ca^{2+}]_i$) from the 10 min platelet sample. The records of other samples were not significantly different.

increase in Ca^{2+}. Since at present only Ins(1,4,5)P_3 has been shown to mobilize intracellular Ca^{2+}, it was decided to compare the time course of inositol polyphosphates with that of Ca^{2+} mobilization. This measurement was made for the experiment shown in Fig. 4(*a*), and is presented in Fig. 4(*b*). It is evident that the cytosolic Ca^{2+} concentration is maintained for a longer time than that of Ins(1,4,5)P_3. The initial increase in Ca^{2+} is well correlated with the increase in Ins(1,4,5)P_3, but the prolonged increase in Ca^{2+} seems to correlate better with the concentration of Ins(1,3,4)P_3.

DISCUSSION

We have used a combination of ^{32}P-labelling, specific removal of interfering metabolites and h.p.l.c. to develop a method to quantify the amounts of Ins(1,4,5)P_3, Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 in stimulated platelets. In the first study that used both h.p.l.c. and [^{3}H]inositol-labelled platelets, only one isomer of InsP_3 was detected (Watson *et al.*, 1984). Labelling with ^{32}P was chosen for our experiments, since labelling with [^{3}H]inositol might not have been satisfactory for detection of all the inositol polyphosphates. The labelling of platelets with [^{3}H]inositol requires either long incubation periods or a large amount of this rather expensive radiochemical to achieve sufficient labelling of the precursor inositol phospholipids. The former condition is a problem, because after long periods human platelets become either

less responsive or totally refractory to stimuli. The present method allows cells to be labelled rather inexpensively and quickly. Furthermore, platelets can be labelled in plasma, where they are most stable. In the present study, the identification of the three inositol polyphosphates was based on their retention time on h.p.l.c., their migration on two-dimensional paper chromatography, and the fact that the ^{32}P-labelled metabolites show similar behaviour to ^{3}H-labelled metabolites in other cell types. However, this assignment must remain somewhat tentative until their structures have been confirmed by additional criteria (e.g. Cerdan *et al.*, 1986). In addition, since we have used extraction with $HClO_4$, it is not certain what proportion of each isomer was originally a 1,2-cyclic form (Ishii *et al.*, 1986). With care, this method should be applicable to the study of inositol polyphosphate metabolism in other types of cells, in particular those with poor uptake of [^{3}H]inositol or cells that must be studied shortly after isolation.

The temporal relationship between the formation of the two InsP_3 isomers and InsP_4 is similar to that measured in other cells. This relationship, and the conversion of Ins(1,4,5)P_3 first into Ins(1,3,4,5)P_4 and then into Ins(1,3,4)P_3, indicate that the metabolic pathways for inositol polyphosphate metabolism are qualitatively the same in platelets as for brain, liver and parotid cells (Irvine *et al.*, 1984, 1985; Burgess *et al.*, 1985; Hawkins *et al.*, 1986; Hansen *et al.*, 1986). The major inositol polyphosphate that accumulates in platelets is Ins(1,3,4)P_3. The K_m for release of Ca^{2+} by Ins(1,4,5)P_3 in permeabilized platelets is about 1 μM (Brass & Joseph, 1985). In two previous studies (Rittenhouse & Sasson, 1985; Dangelmaier *et al.*, 1986), the basal concentrations of InsP_3 were in the range of 1 μM, and an InsP_3 concentration of 15–20 μM was measured for thrombin-stimulated cells. The basal value is obviously too high to maintain a low intracellular Ca^{2+} concentration. The resolution of h.p.l.c. used in the present study allows a better estimate of basal values of Ins(1,4,5)P_3 without interference from other metabolites. We have found that the basal concentration of Ins(1,4,5)P_3 is below the detectable limit of our system. This result is more compatible with the proposed second-messenger role of Ins(1,4,5)P_3. In addition, we can calculate that the maximal concentration of Ins(1,4,5)P_3 reaches about 10% of the total InsP_3, which would be about 2 μM. This value is quite compatible with the determination by Brass & Joseph (1985).

If our estimate of a peak concentration for Ins(1,4,5)P_3 of 2 μM is correct, then the concentrations of Ins(1,4,5)P_3 present after the initial 30–60 s are not sufficient to maintain an elevated intracellular [Ca^{2+}]. However, measurement with fura-2 indicates that intracellular Ca^{2+} is quite high long after this initial period. One explanation is that once Ins(1,4,5)P_3 opens internal Ca^{2+} 'gates', these gates remain open for a prolonged period of time. However, Joseph *et al.* (1984) showed that, in permeabilized hepatocytes, degradation of Ins(1,4,5)P_3 resulted in an immediate fall in Ca^{2+} concentration. Our data indicate that sustained Ca^{2+} concentrations could be correlated with the concentration of either Ins(1,3,4,5)P_4 or Ins(1,3,4)P_3.

In conclusion, the formation and metabolism of Ins(1,4,5)P_3 in human platelets stimulated by thrombin is extremely rapid, but parallels the reaction sequence observed in other cell types. Our data are compatible with a second-messenger role for Ins(1,4,5)P_3, but suggest that other inositol polyphosphates may be important in a sustaining elevated cytoplasmic [Ca^{2+}]. After the initial submission of this manuscript, Irvine *et al.* (1986) showed that Ins(1,3,4)P_3 is able to mobilize Ca^{2+} from intracellular stores in a permeabilized Swiss-mouse 3T3-cell preparation. Half-maximal response was achieved at 9 μM-Ins(1,3,4)P_3. Ins(1,3,4,5)P_4 did not mobilize intracellular Ca^{2+}, but Irvine & Moor (1986) have demonstrated that Ins(1,3,4,5)P_4 can allow influx of extracellular Ca^{2+} when it is injected into sea-urchin eggs. Since we have estimated that the intracellular concentration of Ins(1,3,4)P_3 exceeds 9 μM, we suggest that Ins(1,3,4)P_3 does play an important role in sustaining elevated [Ca^{2+}] in thrombin-stimulated human platelets.

This work was supported by grants HL-14217 and BSRG S07 RR05417 from N.I.H. We thank Dr. Mary Selak for advice and criticism during preparation of this manuscript.

REFERENCES

Aster, R. H. & Jandl, J. H. (1964) J. Clin. Invest. **43**, 834–855
Batty, I. R., Nahorski, S. R. & Irvine, R. F. (1985) Biochem. J. **232**, 211–215
Berridge, M. J. & Irvine, R. F. (1984) Nature (London) **312**, 315–321
Brass, L. F. & Joseph, S. K. (1985) J. Biol. Chem. **260**, 15172–15179
Burgess, G. M., McKinney, J. S., Irvine, R. F. & Putney, J. W. (1985) Biochem. J. **232**, 237–243
Cerdan, S., Hansen, C. A., Johanson, R., Insushi, T. & Williamson, J. R. (1986) J. Biol. Chem. **261**, 14676–14680
Dangelmaier, C. A., Daniel, J. L. & Smith, J. B. (1986) Anal. Biochem. **154**, 414–419
Daniel, J. L., Dangelmaier, C. A., Selak, M. & Smith, J. B. (1986) FEBS Lett. **206**, 299–303
Downes, C. P., Hawkins, P. T. & Irvine, R. F. (1986) Biochem. J. **238**, 501–506
Hansen, C. A., Mah, S. & Williamson, J. R. (1986) J. Biol. Chem. **261**, 8100–8103
Hawkins, P. T., Stephens, L. & Downes, C. P. (1986) Biochem. J. **238**, 507–516
Holmsen, H., Dangelmaier, C. A. & Akkerman, J. W. N. (1983) Anal. Biochem. **131**, 266–272
Irvine, R. F. & Moor, R. M. (1986) Biochem. J. **240**, 917–920
Irvine, R. F., Letcher, A. J., Lander, D. J. & Downes, C. P. (1984) Biochem. J. **223**, 237–243
Irvine, R. F., Anggard, E. E., Letcher, A. J. & Downes, C. P. (1985) Biochem. J. **229**, 505–511
Irvine, R. F., Letcher, A. J., Lander, D. J. & Berridge, M. J. (1986) Biochem. J. **240**, 301–304
Ishii, H., Connolly, T. M., Bross, T. E. & Majerus, P. W. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 6397–6401
Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. & Williamson, J. R. (1984) J. Biol. Chem. **259**, 3077–3081
Meek, J. L. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 4162–4166
Michell, R. H., Kirk, C. J., Jones, L. M., Downes, C. P. & Creba, J. A. (1981) Philos. Trans. R. Soc. London B **296**, 123–137
Nishizuka, Y. (1984) Nature (London) **308**, 693–694
Pollack, W. K., Rink, T. J. & Irvine, R. F. (1986) Biochem. J. **235**, 869–877
Rittenhouse, S. E. & Sasson, J. P. (1985) J. Biol. Chem. **260**, 8657–8660
Rossier, M. F., Dentand, I. A., Lew, P. D., Capponi, A. M. & Vallotton, M. B. (1986) Biochem. Biophys. Res. Commun. **139**, 259–265

Stewart, S. J., Prpic, V., Powers, F. S., Bocckino, S. B., Isaacks, R. E. & Exton, J. H. (1986) Proc. Natl. Acad. Sci. U.S.A. **83**, 6098–6102
Streb, H., Irvine, I. R., Berridge, M. J. & Schultz, I. (1983) Nature (London) **306**, 67–69
Sugino, Y. & Miyoshi, Y. (1964) J. Biol. Chem. **239**, 2360–2364
Watson, S. P., McConnell, R. T. & Lapetina, E. G. (1984) J. Biol. Chem. **259**, 13199–13203
Watson, S. P., Reep, B., McConnell, R. T. & Lapetina, E. G. (1985) J. Biol. Chem. **260**, 831–837

Received 30 December 1986/30 March 1987; accepted 5 May 1987