Blood platelets express high levels of the pp60^{c-src}-specific tyrosine kinase activity

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ABSTRACT We have examined human and rabbit blood platelets for expression of pp60^{c-src}, the normal cellular homolog of the transforming protein of Rous sarcoma virus. pp60^{c-src} kinase activity was determined by an immune-complex kinase assay that uses enolase as the substrate, and pp60^{c-src} protein levels were determined by an immunoblot assay. Lysates from platelets expressed high levels of pp60^{c-src}-specific kinase activity and pp60^{c-src} protein compared to the levels found in other tissues. pp60^{c-src} was also found to be one of the major proteins phosphorylated *in vitro* in membranes isolated from platelets. Multiple protein species other than pp60^{c-src} were also phosphorylated on tyrosine in the membrane phosphorylation reactions, and phosphotyrosine represented $\approx 80\%$ of the total phosphoamino acid residues phosphorylated in the membranes. These results indicate that tyrosine kinases represent the major protein phosphorylating enzymes detected in isolated platelet membranes. Although the association of tyrosine kinase activity with many viral oncogene products and cellular growth hormone receptors has suggested a role for these enzymes in the regulation of cell proliferation, these results indicate that the expression of high levels of tyrosine kinase activity is not exclusively associated with proliferating cells.

The *c-src* gene, which is homologous to the transforming gene, *v-src*, of Rous sarcoma virus (RSV), is highly conserved across widely divergent vertebrate species (1, 2), and closely related genes have been identified in several invertebrate species (3-5). An essential role for the *c-src* gene product is suggested by this conservation; however, the function of this protein in normal cells is not known. Both the *v-src* and *c-src* genes code for phosphoproteins of M_r 60,000 that possess tyrosine-specific kinase activity (for review see ref. 6). The *c-src* protein is present in very low levels in cultured fibroblasts and phosphorylation of tyrosine residues represents a rare event in these cells, accounting for only 0.03% of the total phosphate incorporated into cellular proteins (7). Transformation by most retroviruses encoding tyrosine kinase transforming proteins causes a 7- to 10-fold increase in the levels of phosphotyrosine in cellular phosphoproteins (7). This observation suggests that tyrosine phosphorylation of cellular proteins is involved in transformation, however, the specific substrates that mediate transformation have not been identified, and thus the role of tyrosine kinase activity in transformation is not known (for review see ref. 8). The role of the *c-src* tyrosine kinase activity in normal cells has been equally elusive, and no candidate substrates of the *c-src* kinase activity have been reported. Several investigators have reported the detection of high levels of tyrosine kinase activity in particulate fractions from normal hematopoietic cells (9-13). However, the

kinases responsible for this activity have not been identified. We report here that pp60^{c-src} levels are very high in blood platelets and peripheral blood lymphocytes (PBL). Since *c-src* protein has been found to associate with the plasma membrane (14), we have examined the phosphorylation of proteins in isolated membranes from platelets to determine whether high levels of kinase activity in total cell lysates correlated with elevated levels of tyrosine phosphorylation in membranes. Tyrosine-specific phosphorylation of proteins was found to represent the major kinase activity present in platelet membranes and pp60^{c-src} was one of the most prominent tyrosine phosphorylated species. These results suggest that pp60^{c-src} might serve a specific function in the membranes of platelets and that expression of tyrosine kinase activity is not limited to proliferating cells.

MATERIALS AND METHODS

Tissues and Cells. Blood was taken from human volunteers using 10 mM EDTA as an anticoagulant. After centrifugation of the blood at $1500 \times g$ for 8 min, the buffy coat was removed, layered onto Ficoll/Paque (Pharmacia) gradients and centrifuged as described (15). The lymphocyte layer was removed and washed with balanced salt solution [150 mM NaCl, 10 mM EDTA, 10 mM Tris-HCl (pH 7.5)] to remove contaminating platelets. Platelets were retrieved from these washes and also from the blood plasma (after the removal of the buffy coat) by centrifugation at $2000 \times g$ for 10 min. Erythrocytes were retrieved after the first centrifugation. The purity of different cell fractions was checked by direct microscopic examination. Rabbits were exsanguinated by cardiac puncture, and muscle, brain, spleen, thymus, lymph node, and bone marrow were excised. Lymphocytes, erythrocytes, and platelets were separated from the peripheral blood as described above.

SRD 3T3 cells were derived from RSV (subgroup D) transformation of mouse BALB 3T3 cells. pMc-srcB cells were derived from NIH 3T3 cells by Johnson *et al.* (16) by transformation with a plasmid carrying the chicken *c-src* gene. These cells express 20- to 40-fold higher levels of the *c-src* protein than NIH 3T3 cells.

Immunoprecipitations and *in Vitro* Kinase Reactions. pp60^{c-src} was immunoprecipitated from lysates with 0.01 μ l of monoclonal antibody (mAb) 327 (obtained from ascites fluid) by using formalin-fixed *Staphylococcus aureus* bacteria as described (17). *In vitro* kinase reactions were performed after immunoprecipitation of the pp60^{c-src} by the addition of 2 μ g of acid-treated enolase (18), 5 μ Ci of [γ -³²P]ATP (1 Ci = 37 GBq), 1 μ M ATP, 10 mM Tris-HCl (pH 7.2), and 5 mM MgCl₂ for 15 min at room temperature. The reactions were stopped with the addition of NaDodSO₄ sample buffer and then subjected to electrophoresis on 7.5% NaDodSO₄/polyacrylamide gels as described by Laemmli (19). The dried gels were

then exposed to XAR-5 film (Kodak) and enhanced with Cronex Lightning Plus intensifying screens.

Immunoblot Assay. Immunoprecipitated pp60^{c-src} was subjected to electrophoresis as above and the 60-kDa region of the gel was electroblotted onto nitrocellulose filter paper (20). The filter was then incubated with mAb 327 followed with ¹²⁵I-labeled antimouse immunoglobulins. All antibody reactions were performed in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.5% Tween 20. pp60^{v-src} expressed in *Escherichia coli* (21) was used as a marker.

Membrane Isolation and *in Vitro* Phosphorylation. Membrane preparation from human platelets and erythrocytes was carried out as described by Thom *et al.* (22), including 10 μ M sodium orthovanadate (Na₃VO₄) in all buffers and employing an additional step of Dounce homogenization of the membrane pellet before layering onto the sucrose cushion. The use of nylon gauze was omitted.

Each membrane protein preparation was incubated 15 min at room temperature with 50 μ Ci [γ -³²P]ATP, 5 μ M ATP, 5 mM MgCl₂, 10 mM Hepes (pH 7.4), and 10% (vol/vol) glycerol. Reactions were stopped with NaDodSO₄ sample buffer. Alkali treatment of gels was carried out as described by Cooper and Hunter (23).

Phosphoamino Acid Analysis. Phosphoproteins were eluted from the gels by digestion with 50 μ g of trypsin in 1 ml of 0.05 M ammonium carbonate, pH 8.5. After lyophilization, samples were hydrolyzed with 6 M HCl for 1.75 hr as described (24). Phosphoamino acid analysis was performed on Whatman Grade No. 3MM Chrom paper for 1.75 hr at 4000 V at pH 3.5. After autoradiography, the spots were excised and solubilized, and the amount of radioactivity in each spot was determined.

***In Vivo* Labeling of Platelets.** Approximately 10⁹ platelets were isolated from freshly drawn human blood as described earlier and washed with platelet labeling medium (150 mM NaCl/15 mM Tris-HCl, pH 7.4/5.5 mM glucose) (25). Cells were labeled with [³²P]orthophosphate (ICN, 1 mCi/ml) *in vivo* in platelet labeling medium for 1 hr at 37°C. Cells were then pelleted, washed twice with phosphate-buffered saline [150 mM NaCl, 2.5 mM KCl, 1.5 mM KH₂PO₄, 10 mM Na₂HPO₄ (pH 7.4)], and cell lysates were prepared for pp60^{c-src} immunoprecipitation as described above.

Partial Proteolytic Peptide Analysis. *In vitro* and *in vivo* phosphorylated proteins were localized by autoradiography,

excised from the gel, and subjected to electrophoresis on 12.5% NaDodSO₄/polyacrylamide gels in the presence of 150 ng of V8 protease (Miles) following the procedure developed by Cleveland *et al.* (26).

RESULTS

High Levels of pp60^{c-src}-Specific Kinase Activity in Rabbit and Human Platelets and Lymphocytes. To examine the expression of the c-src protein in hematopoietic cells, we compared the levels of pp60^{c-src}-associated kinase activity in various peripheral blood cells and tissues from an adult rabbit. Lymphocytes, erythrocytes, and platelets were separated from peripheral blood and the following tissues were excised: spleen, thymus, lymph node, bone marrow, brain, and muscle. Fig. 1A shows the phosphorylation of enolase after immunoprecipitation of pp60^{c-src} from cell lysates with a monoclonal antibody directed against the src protein (17). The relative level of enolase phosphorylation per microgram of total cell protein was quantified for each lysate. Platelet lysates contained the highest levels of pp60^{c-src}-specific enolase phosphorylation, 3- to 7-fold higher than the levels detected in brain and peripheral blood lymphocyte (PBL) lysates and at least 30- to 50-fold higher than muscle, spleen, thymus, lymph node, bone marrow, and erythrocyte lysates.

In addition to enolase phosphorylation, we also detected proteins of *M*_r 60,000 that were highly phosphorylated in platelets and PBL. To determine whether these proteins were pp60^{c-src} and to examine the site(s) of phosphorylation on these proteins, they were subjected to partial proteolytic cleavage with V8 protease (Fig. 2A, lanes 3 and 4). The cleavage patterns were compared to that of a pp60^{c-src} marker (16) labeled *in vivo* with [³²P]orthophosphate (Fig. 2A, lane 1). Fig. 2B shows the V8 cleavage pattern of ³²P-labeled pp60^{c-src}. Cleavage of pp60^{c-src} with low concentrations of V8 protease results in cleavage at a single site that separates the pp60^{c-src} protein into an amino-terminal V1 fragment (*M*_r 34,000) and a carboxyl-terminal V2 fragment (*M*_r 26,000). Further digestion with V8 protease cleaves the V1 fragment to generate a V3 fragment (*M*_r 18,000) and a V4 fragment (*M*_r 16,000). It was shown previously that the V2 fragment of pp60^{c-src} is exclusively phosphorylated *in vitro* (27), presumably on tyrosine-416 (28). Fig. 2A shows that the fragments derived from the partial proteolytic cleavage of the pp60 bands phosphorylated *in vitro* in platelets and PBL (Fig. 1A,

FIG. 1. (A) Proteins phosphorylated *in vitro* following immunoprecipitations from lysates of various normal rabbit and human tissues and cells. Immunoprecipitates prepared from lysates using mAb 327 and rabbit anti-mouse IgG (even numbered lanes) or rabbit anti-mouse alone (odd numbered lanes) were assayed for enolase phosphorylating activity. Lanes: 1 and 2, 250 μ g of muscle lysate; 3 and 4, 250 μ g of brain lysate; 5 and 6, 375 μ g of PBL lysate; 7 and 8, 180 μ g of platelet lysate; 9 and 10, 2500 μ g of erythrocyte lysate; 11 and 12, 500 μ g of spleen lysate; 13 and 14, 250 μ g of thymus lysate; 15 and 16, 500 μ g of lymph node lysate; 17 and 18, 500 μ g of bone marrow lysate; 19 and 20, 100 μ g of human PBL lysate; 21 and 22, 100 μ g of human platelet lysate; and 23 and 24, 100 μ g of SRD/3T3 cell lysate. The autoradiograph for lanes 1-18 was exposed for 4.5 hr, lanes 19-24, 7 hr. The 52-kDa protein band in lanes 20 and 22 is a proteolytic cleavage product of pp60^{c-src} generated during cell lysis. (B) pp60^{c-src} was immunoprecipitated from 1500 μ g of protein from lysates of rabbit tissues using mAb 327 as described (17). After electrophoresis, the 60-kDa region of the gel was transferred to nitrocellulose and pp60^{c-src} was detected. Lanes: 1, bacterial src protein; 2, pMc-srcB; 3, muscle; 4, brain; 5, platelets; 6, SRD/3T3; 7, erythrocytes; 8, spleen; 9, thymus; 10, bone marrow; and 11, PBL.

FIG. 2. Peptide analysis of M_r 60,000 phosphoproteins. (A) The M_r 60,000 phosphorylated proteins were excised from Fig. 1 (lanes 6 and 8) and Fig. 3 (lane 3, band L) and subjected to electrophoresis in the presence of 150 ng of V8 protease as described (26). Lanes: 1, pp60^{c-src} immunoprecipitated with mAb 327 from mouse pMc-srcB cells (16) (this protein species was used as a pp60^{c-src} marker); 2, pp60^{c-src} immunoprecipitated with mAb 327 from human platelets labeled *in vivo* with [³²P]orthophosphate; 3, pp60^{c-src} phosphorylated *in vitro* after immunoprecipitation from rabbit platelet lysate with mAb 327 (Fig. 1, lane 8); 4, pp60^{c-src} phosphorylated *in vitro* after immunoprecipitation from rabbit PBL lysate with mAb 327 (Fig. 1, lane 6); 5, phosphoprotein of M_r 60,000 from an *in vitro* phosphorylated platelet membrane preparation (Fig. 3, lane 3, protein band L). (B) *Staphylococcus aureus* V8 protease cleavage map of pp60^{c-src}.

lanes 6 and 8) comigrated with the V2 fragment of the pp60^{c-src} marker. Since the mAb used in this study does not crossreact with any of the viral oncogenes closely related to the *src* gene, these results provide evidence that the 60-kDa proteins phosphorylated in these reactions are pp60^{c-src}. It is of interest to note that the pp60^{c-src} protein from rabbit brain lysates consistently showed lower levels of autophosphorylation relative to enolase phosphorylation than the c-src protein from platelets and lymphocytes.

To compare the level of activity of the c-src protein in platelets to that of the v-src protein in RSV-transformed cells, pp60^{c-src}-specific enolase phosphorylation was examined from RSV-transformed mouse cells (SRD 3T3 cells), human peripheral blood platelets, and the PBL fraction from Ficoll-density gradients (Fig. 1A, lanes 19–24). The levels of enolase phosphorylation in all three cell types were similar, although the level of pp60^{c-src} autophosphorylation was higher in platelets than in cells from the PBL fraction and higher than the level of pp60^{v-src} autophosphorylation in RSV-transformed cells.

To determine whether these differences in pp60^{c-src}-specific kinase activity reflected differences in the level of the *src* protein in platelets and other tissues, we immunoprecipitated the *src* protein from 1500 μ g of protein lysate from various rabbit tissues and platelets, as well as from RSV-transformed cells and pMc-srcB cells, which overexpress the c-src protein. The levels of pp60^{c-src} protein were determined by an immunoblot assay. Fig. 1B shows that platelets (lane 5) contained the highest levels of pp60^{c-src}, slightly higher than cells from the PBL fraction (lane 11) and brain (lane 4) and approximately 5-fold higher than SRD 3T3 cells (lane 6) and

the mouse cells that overexpress c-src (lane 2). The c-src protein was undetectable from the other lysates in this assay.

High Levels of Tyrosine Residue Phosphorylation in Platelet Membranes. Since the levels of pp60^{c-src}-specific kinase activity are higher in platelet lysates than in any other cell lysates, it was of interest to examine the contribution of tyrosine-specific kinase activity to the phosphorylation of membrane proteins. Platelet and erythrocyte membranes were isolated in the presence of sodium vanadate to inhibit phosphotyrosine-specific phosphatases (10, 29) and were incubated with [³²P]ATP to assay phosphorylation of membrane proteins. Duplicate reaction mixes were electrophoresed on NaDodSO₄/polyacrylamide gels, and one set was treated with alkali (1 M KOH). This treatment was performed to hydrolyze the phosphoserine and phosphothreonine residues, since the phosphodiester linkage to tyrosine residues is known to be more resistant to alkali treatment than phosphothreonine and phosphoserine (23, 30, 31). The results of this experiment are shown in Fig. 3. Multiple protein species were phosphorylated in the platelet membrane preparation (lane 3). Most of these protein bands were alkali insensitive (lane 7), suggesting that phosphorylation of tyrosine residues was extensive. Membranes prepared in the absence of sodium vanadate exhibited a similar pattern of phosphorylation (data not shown). One of the major phosphorylated, alkali-resistant bands comigrated with a pp60^{c-src} marker. To determine if this pp60 band (Fig. 3, lane 3, band L) was pp60^{c-src}, this protein band was excised from the gel and subjected to peptide analysis (Fig. 2A, lane 5). Partial proteolytic digestion with V8 protease yielded a single peptide that comigrated with the V2 fragment of the pp60^{c-src} marker. This result provides evidence that this *in vitro* phosphorylated M_r 60,000 protein found in the platelet membrane is the c-src protein and is phosphorylated exclusively within the carboxyl-terminal 26 kDa of the protein.

FIG. 3. Membrane phosphorylation. Membranes extracted from platelets (lanes 3 and 7) and erythrocytes (lanes 1 and 5) in the presence of 10 μ M sodium vanadate were incubated with [³²P]-ATP. Identical membrane preparations from platelets (lanes 4 and 8) and erythrocytes (lanes 2 and 6) were phosphorylated as above in the presence of 0.5% Triton X-100. Lanes 1–4, nonalkali treatment; lanes 5–8, alkali treatment. Bands labeled A through G (lane 1) are the erythrocyte phosphoproteins that correspond to samples A through G in Fig. 4. Bands labeled H through O (lane 3) are the platelet phosphoproteins that correspond to samples H through O in Fig. 4. Some of the bands labeled represent bands that were more clearly identified in a longer exposure of the gel. The pp60^{c-src} band corresponds to the band labeled L. Platelet membranes (45.0 μ g) were used in every platelet phosphorylation reaction and 57.5 μ g of erythrocyte membranes were used in every erythrocyte phosphorylation reaction.

The two major phosphorylated protein bands (Fig. 3, lane 1, bands A and C) detected in the erythrocyte membrane phosphorylation reaction were found to be somewhat alkali insensitive (lane 5) in this experiment. However, when the same samples were subjected to electrophoresis on a thinner gel, all of the ^{32}P from the high molecular weight protein (band A) was hydrolyzed and $\approx 80\%$ of the 93-kDa phosphoprotein (band C) was hydrolyzed. Other investigators have reported a 93-kDa protein that is phosphorylated on tyrosine residues in erythrocyte membranes (32).

Since tyrosine kinase activity associated with cellular membrane fractions has been shown to be stimulated by nonionic detergents (10, 12), we incubated our membranes with Triton X-100 (Fig. 3, lanes 2, 4, 6, and 8). This treatment enhanced the phosphorylation of the pp60 protein band and some of the lower molecular weight protein bands of the platelet membranes in lane 4 of Fig. 3. Interestingly, there was no appreciable change in the erythrocyte membrane phosphorylation pattern upon incubation with Triton X-100 (Fig. 3, lane 2).

Since alkali resistance is highly suggestive of tyrosine residue phosphorylation, phosphoamino acid analysis was carried out on the seven most prominent erythrocyte phosphoproteins and the eight most prominent platelet phosphoproteins from the nonalkali treated gels in Fig. 3 (lanes 1 and 3, respectively). The results of the phosphoamino acid analysis of these platelet phosphoproteins, shown in Fig. 4, indicated that all eight proteins excised from the gel contained predominantly phosphotyrosine. Quantitation of the radioactivity in each spot revealed that tyrosine phosphorylation represented 78% of the total phosphoamino acids in the platelet bands analyzed. Tyrosine residue phosphorylation in the erythrocyte membranes accounted for only 13% of the total phosphoamino acids, most of which were derived from the 93-kDa phosphoprotein (Fig. 3, lane 1, band C).

Analysis of pp60^{c-src} Labeled *in Vivo* with ^{32}P . We have shown (33, 34) that the pp60^{c-src} protein present in cultured rat neurons possesses a higher specific activity than the pp60^{c-src} protein expressed in other cell types. This elevation in the pp60^{c-src} kinase activity from neurons was found to correlate with the presence of a structural modification within the amino-terminal region of the molecule that caused a shift in the electrophoretic mobility of pp60^{c-src} and peptides derived from the modified region (P. C. Cotton and J.S.B., unpublished results). To determine whether pp60^{c-src} from platelets was modified in a similar fashion, we labeled the platelets with [^{32}P]orthophosphate *in vivo* and immunoprecipitated ^{32}P -labeled pp60^{c-src} from cell lysates using mAb 327. A single ^{32}P -labeled protein of M_r 60,000 was specifically immunoprecipitated from the labeled platelets (data not shown). This protein species was subjected to peptide analysis by using V8 protease. The results, shown in Fig. 2A (lane 2), confirm that the M_r 60,000 phosphoprotein immunoprecipitated from *in*

vivo labeled platelets is the c-src gene product. The V1, V2, V3, and V4 peptides of platelet pp60^{c-src} comigrated with the V8 cleavage fragments of the pp60^{c-src} marker, indicating that pp60^{c-src} from platelets is not modified as in the neurons. The detection of ^{32}P incorporation into the V1, V2, V3, and V4 peptide fragments indicates that the pp60^{c-src} is phosphorylated within the amino- and carboxyl-terminal halves of the protein *in vivo*.

DISCUSSION

We have shown that the levels of c-src-specific kinase activity are elevated in blood platelets and that the c-src protein represents one of the major phosphorylated protein species in our platelet membrane phosphorylation reactions. We have also found multiple phosphorylated protein species, other than the c-src protein that are phosphorylated extensively on tyrosine residues. These phosphorylated protein species could represent candidate substrates of pp60^{c-src}-specific kinase activity or other tyrosine kinases present in platelet membranes. This high level of c-src-specific kinase activity correlates with an increase in the levels of c-src protein in platelets. These results suggest that tyrosine residue phosphorylation accounts for the major kinase activity in platelet membranes isolated under the conditions of these assays, and thus raise the question of whether tyrosine residue phosphorylation may be involved in essential platelet membrane functions.

Other investigators (9–13) have reported the detection of tyrosine kinase activity in normal hematopoietic cells including platelets, erythrocytes, lymphocytes, and a T-cell lymphoma cell line (LSTRA). Tuy *et al.* (9) have reported high tyrosine kinase activity in the particulate fraction of platelets and detected an alkali-resistant 60-kDa phosphoprotein. They concluded from V8 protease peptide analysis that this protein was not the c-src protein. In comparing the gels from the report of Tuy *et al.* with those from this study, it appears that the gels employed in their study would not have resolved the proteins of M_r 60,000 and 58,000 that were detected in Fig. 3 (lane 3, bands L and M). Thus, it is quite possible that both these proteins were subjected to proteolytic digestion in the analysis by Tuy *et al.*, thus confusing the V8 protease cleavage profile. Earp *et al.* (12) have reported that particulate fractions from B lymphocytes possess two alkali-resistant phosphoproteins of M_r 55,000 and 61,000 and that particulate fractions from T lymphocytes possess two alkali-resistant phosphoproteins of M_r 58,000 and 64,000. Phosphorylation of tyrosine residues was common to all four proteins. In another report, Harrison *et al.* (13) detected two phosphoproteins of M_r 56,000 and 60,000 in the particulate fraction of B lymphocytes. In these studies the investigators did not establish whether these phosphotyrosine-containing proteins possessed intrinsic tyrosine kinase activity, whether these proteins are substrates of tyrosine kinases, or whether they are structurally related to each other or previously identified tyrosine kinases. It is possible that the 60-kDa phosphoprotein detected in B cells in the report by Harrison *et al.* and the 61-kDa protein detected in B cells by Earp *et al.* could be the c-src protein.

We have also detected high levels of pp60^{c-src} in the lymphocyte fraction from peripheral blood, however, we have not established which type of PBL possesses elevated levels of the c-src protein kinase activity. It was surprising to detect low levels of activity in bone marrow, spleen, thymus, and lymph node lysates in our studies since these tissues contain large populations of lymphocytes. It is possible that a minor lymphocyte population in the spleen, for example, contains high levels of the pp60^{c-src}-specific kinase activity, but that its signal is diluted out by the majority of splenic cells that lack this kinase activity. All of these results indicate that

FIG. 4. Phosphoamino acid analysis of phosphoproteins from erythrocyte and platelet membranes. Bands A through O were excised from the nonalkali treated gel in Fig. 3 (lanes 1 and 3) and subjected to phosphoamino acid analysis. P-Ser, phosphoserine; P-Thr, phosphothreonine; P-Tyr, phosphotyrosine.

hematopoietic cells, in general, contain high levels of tyrosine kinase activity, and thus provide evidence that appreciable amounts of tyrosine-specific protein kinase activity are present in certain normal mammalian cells.

The level of c-src protein kinase activity detected in platelet lysates was comparable to the levels of activity found in RSV-transformed mouse cells (Fig. 1A, lanes 22 and 24). Since the immunoblot assay (Fig. 1B, lanes 5 and 6) indicated that platelets contain approximately 5-fold higher levels of the c-src protein per microgram of total cell protein than the levels of the v-src protein in RSV-transformed cells, this result suggests that the specific activity of the platelet c-src protein is 20% that of the v-src protein in RSV-transformed cells. This high level of activity is similar to that observed for pp60^{c-src} expressed in neurons and significantly higher than the activity of the c-src protein expressed in chicken fibroblasts or mouse 3T3 cells, which has been reported to have a specific activity 2–5% that of v-src (35, 36).

It is likely that the c-src protein is enriched in the plasma membrane of platelets. We have found that the highest levels of the c-src protein were detected in the least dense membrane fraction when the membranes were fractionated on sucrose gradients after isolation by either the Thom procedure employed in this report or by the homogenization method (III) described by Day *et al.* (37). This fraction also contained the highest levels of the two plasma membrane markers, 5'-nucleotidase and Na-K ATPase (data not shown). Through this series of membrane fractionation experiments, we are confident that the c-src protein and its kinase activity are enriched in the plasma membrane, but we cannot, as yet, eliminate the possibilities that other platelet membranes possess the elevated c-src activity also.

The platelet is unique in that it is a nonproliferating, anuclear cell that is formed by the fragmentation of its precursor cell, the megakaryocyte, rather than by maturation of a more primitive cell. Each megakaryocyte divides in the bone marrow and produces a total of ≈ 2000 platelets (38). We have been fortunate to have obtained megakaryocytic cell lines (39) from Doris Morgan at Hahnemann University, and we have preliminary evidence that these cell lines also possess elevated levels of the pp60^{c-src}-specific kinase activity.

The levels of pp60^{c-src}-specific kinase activity in platelets are comparable to the levels observed in transformed mammalian cells. The detection of these elevated levels of kinase activity in normal blood platelets indicates that high levels of tyrosine kinase activity do not correlate with cell proliferation, but may actually be involved in other cellular functions not exclusively related to growth control. This possibility is also suggested by the finding that postmitotic neurons also contain elevated levels of pp60^{c-src} kinase activity. One possible function of the c-src protein and other tyrosine kinases may involve the transduction of extracellular signals. It is clear that the primary function of at least one class of tyrosine kinases, the membrane receptors for the growth hormones, platelet-derived growth factor (40, 41), epidermal growth factor (42), and insulin (43, 44), serves to transmit extracellular signals to their respective cells.

Platelets could provide an ideal system in which to investigate the functions of tyrosine kinases since the physiological responses to a variety of extracellular stimuli have been well characterized, and the levels of pp60^{c-src} are at least as high as those found in RSV-transformed cells.

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