# Platelet endothelial cell adhesion molecule-1 is a major SH-PTP2 binding protein in vascular endothelial cells

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**Abstract Platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is rapidly tyrosine phosphorylated in mechanically stimulated vascular endothelial cells (ECs). A 65-kDa protein from ECs specifically bound to the c-Src phosphorylated PECAM-1 cytoplasmic domain and was identified as a protein tyrosine phosphatase SH-PTP2 (SHP2, Syp). PECAM-1 was coimmunoprecipitated by anti-SH-PTP2 from EC extracts as a major binding protein, and the level of association increased when PECAM-1 was tyrosine phosphorylated. This association was mediated by SH2 domains of SH-PTP2. A rapid translocation of SH-PTP2 into cell-cell adhesion sites, where PECAM-1 was localized, occurred in mechanically stimulated cells. Our results suggest that PECAM-1 is a component of a mechanosensing machinery acting upstream of SH-PTP2.**



*Key words:* PECAM-1; SH-PTP2; Tyrosine phosphorylation; Osmotic shock; Mechanical stimuli; Endothelial cell

## 1. Introduction

Molecular mechanisms for sensing and subsequent signal transduction of mechanical stimuli are largely unknown. Vascular endothelial cells (ECs) are constantly exposed to mechanical forces, fluid shear stress and stretch, from flowing blood. These cells are thought to be able to sense both the magnitude and the direction of fluid flow [1,2]. Both in vivo and in vitro studies have revealed that EC responses to flow include changes in the secretion of vasoactive substances, gene expression and the cytoskeleton and cellular morphology (see [3] for a review). Recently, we found that a 128-kDa cell surface glycoprotein was tyrosine phosphorylated within a min in ECs exposed to fluid flow, hyper- or hypo-osmotic shock and that this phosphorylation was independent of $Ca^{2+}$ mobilization or PKC activation in these cells [4]. The 128-kDa glycoprotein was then identified as platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) [5,6]. Our in vitro data suggested an involvement of Src family kinases in this PECAM-1 tyrosine phosphorylation [6]. PECAM-1 is an immunoglobulin superfamily cell adhesion molecule [7] and is involved in cell-cell adhesion/recognition by homophilic and heterophilic binding mechanisms [8–11]. In ECs, PECAM-1 is concentrated at the region of cell apposition between neighboring cells [8,9]. Recently, PECAM-1 tyrosine dephosphorylation was reported to take place during integrin mediated cell spreading onto solid substrates [12]. This and our data suggest that the levels of PECAM-1 tyrosine phosphorylation is regulated by mechanical forces exerted to cells.

Protein tyrosine phosphorylation plays principal roles in intracellular signaling and regulates various cellular functions. To characterize PECAM-1 mediated signaling, it is crucial to find out proteins specifically interacting with tyrosine phosphorylated PECAM-1. Here, we show that tyrosine phosphorylated PECAM-1 is a major binding protein of SH-PTP2 (also known as PTP1D, PTP2C, Syp, SHP2), a non-transmembrane protein tyrosine phosphatase containing two Src homology 2 (SH2) domains [13], in mechanically stimulated ECs. SH-PTP2 is known to act as a positive signaling molecule just downstream of receptor tyrosine kinases [14–17] and to activate Ras in the MAP kinase cascade [15,17]. Our findings suggest that one of the pathways for the activation of MAP kinases observed in mechanically stimulated ECs [18–21] is mediated by the PECAM-1/SH-PTP2 interaction.

## 2. Materials and methods

### 2.1. Reagents and cells

GST fusion proteins containing the cytoplasmic domain of bovine PECAM-1 (amino acid residues 625–738, GST-PECAM-1Cyt) and its spliced-out form (residues 625–702/722–738, GST-PECAM-1Cyt-ΔEx14) were synthesized [6] using a pGEX-2TK vector (Pharmacia Biotech). GST fusion proteins containing two SH2 domains of SH-PTP2, the SH2 domain of Fyn kinase, the SH2 domain of the PI3 kinase p85α subunit, and the SH2-SH2-SH3 containing domain of PLCγ1 were obtained from Santa Cruz Biotechnology. Affinity purified antibodies against different regions of bovine PECAM-1 were described earlier [6]. Anti-SH-PTP2 (the C-terminus region) was the product of Santa Cruz Biotechnology. Anti-phosphotyrosine (clone 4G10) was obtained from Upstate Biotechnology. c-Src and the catalytic subunit of cyclic AMP dependent kinase (A-kinase) were obtained from Upstate Biotechnology and Sigma, respectively.

Bovine arterial ECs (BAECs) were cultured and stimulated by hyperosmotic condition as described previously with a slight modification [4,6]. Briefly, cells were starved for 24–36 h in a low serum medium (DMEM/F12 containing 1% serum), preincubated for 2 min with 1 mM $Na_3VO_4$ in the same medium and treated for 3 min either with the same vanadate containing medium (control) or the same medium containing 0.3 M sucrose (hyperosmotic medium). Cells were washed with ice-cold PBS containing 1 mM $Na_3VO_4$ and lysed in an ice-cold 50 mM Tris-HCl buffer, pH 7.3, containing 50 mM NaCl, 20 mM NaF, 2 mM EGTA, 2 mM EDTA, 1 mM $Na_3VO_4$, 1 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml E64 and 0.4% Triton X-100 (lysis buffer). The lysate was centrifuged at 15000×g for 20 min, and the supernatant (approximately 1.2 mg/ml protein) was used for assays.

### 2.2. Far Western assay

The lysate was separated on a 10% SDS-PAGE gel, transferred onto a PVDF membrane, and the membrane was blocked by Tris-buffered saline containing 5% nonfat dry milk, 1 mg/ml of GST and 0.02% Tween 80, and then probed with 100 μg of radio-labeled GST or GST-PECAM-1Cyt in the blocking solution. A Bioimage analyzer (BAS2000, Fuji Film) was used to visualize bound radioactive signals. GST and the fusion protein were labeled by A-kinase-catalyzed serine

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phosphorylation or c-Src-dependent tyrosine phosphorylation using [$\gamma$-$^{32}$P]ATP (7000 Ci/mole, add 100 μCi/100 μg protein in 100 μl of the reaction mixture) as the donor. GST was not tyrosine phosphorylated by c-Src in our labeling condition (1 h at 4°C).

*2.3. Affinity purification and microsequencing of a c-Src phosphorylated GST-PECAM-1Cyt binding protein*

Thirty μg of GST-PECAM-1Cyt bound to glutathione-Sepharose 4B beads were phosphorylated by 37 units of c-Src overnight at 4°C in 50 mM HEPES buffer, pH 7.2, containing 50 mM NaCl, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM DTT, 100 μM $Na_3VO_4$ and 1 mM ATP. The beads were extensively washed with Tris-buffered saline containing 10 mg/ml phenylphosphate and 10 mM EDTA to remove c-Src and then incubated with the BAEC lysate obtained from $1\times10^9$ cells. The bound material was eluted with 15 mM glutathione in 100 mM Tris-HCl buffer, pH 8.0, and analyzed on a 10% SDS-PAGE gel. A 65-kDa band, which bound specifically to the affinity column, was excised and the protein was digested in-gel [22] by TPCK-trypsin (Worthington Biochemical Corp.) after reductive carboxymethylation. Peptide fragments were separated by reverse phase HPLC (μRPC C2/C18 SC 2.1/10 column, Smart System, Pharmacia Biotech), and isolated peptides were sequenced by automated Edman degradation using a 492 protein sequencer (Applied Biosystems).

*2.4. Coimmunoprecipitation*

The BAEC lysate, precleaned with 50 μl of protein A Sepharose beads per 1.5 ml lysate, was incubated with 5 μg of anti-PECAM-1 external domain or anti-SH-PTP2 for 1 h at 4°C and further incubated for 1 h with 10 μl of protein A Sepharose beads. After washing with the lysis buffer, the bound material was recovered in SDS-PAGE sample buffer, separated on 8% SDS-PAGE gels and electro-blotted to PVDF membranes. Blotted membranes were probed with chick anti-PECAM-1, anti-SH-PTP2 or anti-phosphotyrosine. Biotinylated secondary antibodies and [$^{125}$I]streptavidin (Amersham) were used to visualize bound antibodies by a Bioimage analyzer.

*2.5. GST-SH2 binding assay*

Glutathione-Sepharose beads preincubated with 3 μg of each GST fusion protein containing SH2 and/or SH3 domains were incubated with 1 ml of the BAEC lysate for 1 h at 4°C. After 5× washing with the lysis buffer, the bound material was recovered in 100 μl of 15 mM glutathione in 100 mM Tris-HCl buffer, pH 8.0, containing 10 mg bovine serum albumin and 0.1% sodium deoxy cholate, and then precipitated by 10% trichloro acetic acid. The precipitated material was used for an immunoblot assay using anti-PECAM-1 as the probe.

*2.6. Confocal laser scanning microscope*

Localization of SH-PTP2 in BAECs stimulated or non-stimulated by the hyperosmotic condition was examined by indirect immunofluorescence microscopy using a confocal laser scanning microscope (GB200, Olympus). BAECs were fixed in MeOH for 15 min at −20°C, blocked with PBS containing 5% of nonfat dry milk, fish gelatin (Sigma) and normal goat serum, and stained with anti-SH-PTP2 or anti-PECAM-1 cytoplasmic domain.

## 3. Results

*3.1. Tyrosine phosphorylated PECAM-1 cytoplasmic domain specifically interact with a 65-kDa protein in vitro*

Far Western blotting was used to find proteins specifically interacting with tyrosine phosphorylated PECAM-1. A GST-PECAM-1 cytoplasmic domain fusion protein, which had been phosphorylated by exogenous c-Src or A-kinase was used as the probe. The c-Src phosphorylated fusion protein bound to a 65-kDa band in the total BAEC lysate (Fig. 1A). However, when GST or GST-PECAM-1Cyt was serine phosphorylated by A-kinase in the linker region of the fusion protein, no detectable binding occurred. Since GST itself

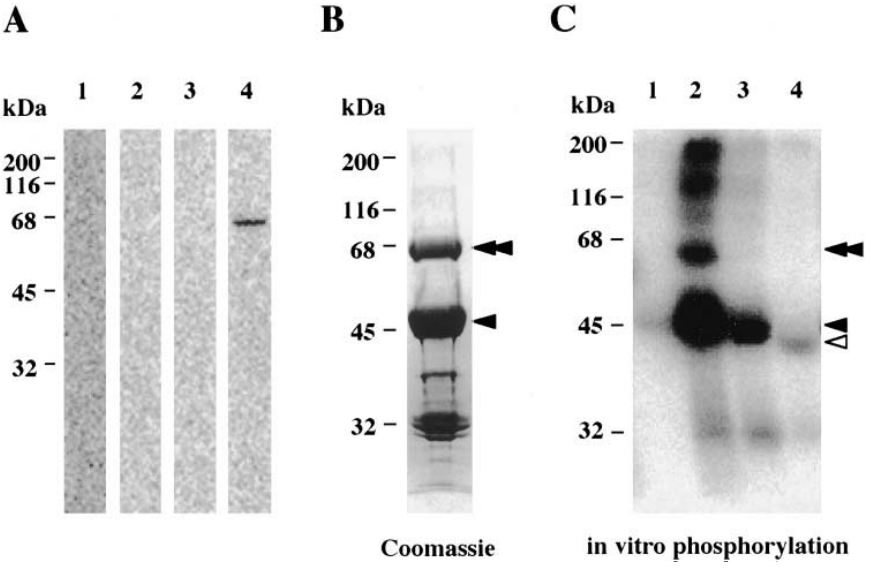


Fig. 1. A 65-kDa protein specifically binds to the tyrosine phosphorylated PECAM-1 cytoplasmic domain. (A) A detergent soluble fraction of non-stimulated BAECs is analyzed by Far Western blotting using a GST fusion protein containing the PECAM-1 cytoplasmic domain as the probe. A-kinase phosphorylated GST (lane 1), A-kinase phosphorylated GST-PECAM-1Cyt (lane 2), c-Src phosphorylated GST (lane 3) and c-Src phosphorylated GST-PECAM-1Cyt (lane 4). (B) Proteins bound to the c-Src phosphorylated GST-PECAM-1Cyt affinity matrix. A total lysate from non-stimulated BAECs ($1\times10^9$ cells) was applied onto the matrix and bound proteins were resolved on a 10% SDS-PAGE gel and stained with Coomassie blue. Almost exclusive binding of 65-kDa polypeptide (double arrowheads) is apparent. This band was used for a microsequencing analysis. The 45-kDa band (arrowhead) is GST-PECAM-1Cyt and the lower molecular weight bands (30–40 kDa) are its proteolytic digests. (C) An in vitro autophosphorylation analysis of materials bound to c-Src phosphorylated GST-PECAM-1Cyt (lanes 1 and 2, the band position is indicated by an arrowhead), non-phosphorylated GST-PECAM-1Cyt (lane 3) and non-phosphorylated GST-PECAM-1Cyt-ΔEx14 (lane 4, white arrowhead). Six and a half mg of the total detergent soluble fraction from non-stimulated BAECs were applied onto the affinity columns, each containing 10 μg of one of the fusion proteins (lanes 2–4). Lane 1 shows the remaining c-Src activity in the phosphorylated GST-PECAM-1Cyt after an extensive wash with phenylphosphate containing buffer. The bound materials were phosphorylated for 20 min at 37°C using [$\gamma$-$^{32}$P]ATP (30 μCi/sample, 100 μM cold ATP), resolved on a 10% SDS-PAGE gel after elution, and the gel was autoradiogramed. The 65-kDa protein (double arrowheads) bound specifically to the c-Src phosphorylated PECAM-1 cytoplasmic domain and was phosphorylated in vitro.

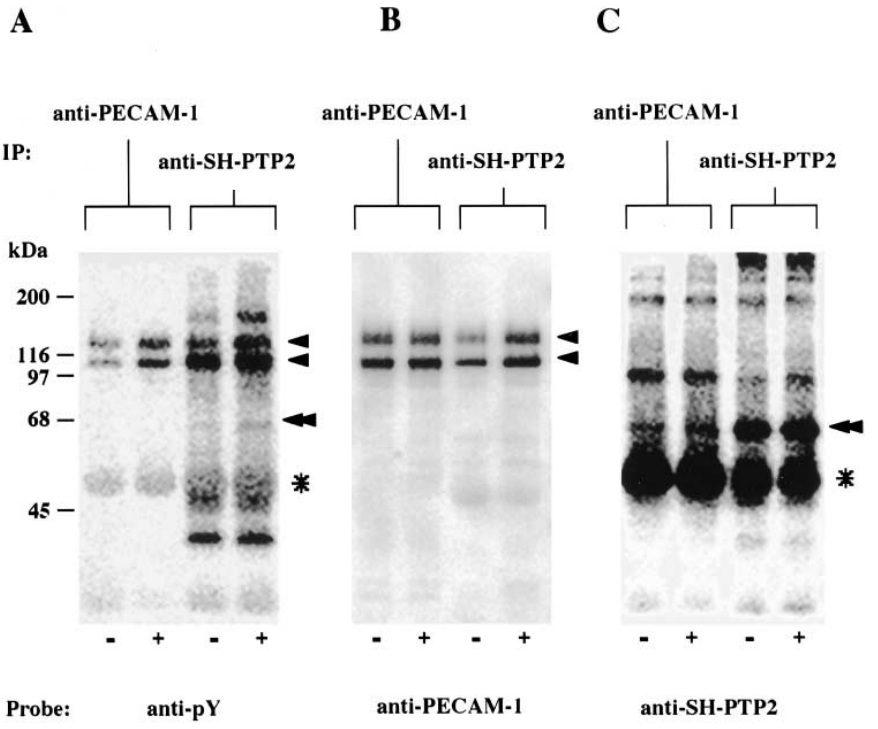


Fig. 2. PECAM-1 is an SH-PTP2 binding protein in BAECs. Immunoprecipitates from a BAEC lysate using anti-PECAM-1 or anti-SH-PTP2 were probed with anti-phosphotyrosine (A), anti-PECAM-1 (B) or anti-SH-PTP2 (C). The same amount of PECAM-1 or SH-PTP2 was recovered from non-stimulated control cells (−) and osmotically stimulated cells (+) (see reprobed lanes in B and C), while the levels of tyrosine phosphorylation of both PECAM-1 and SH-PTP2 (A) and the efficiency of coimmunoprecipitation (B and C) increased when cells were exposed to the hyperosmotic condition for 3 min. Positions of two PECAM-1 bands, the SH-PTP2 band and Ig heavy chain are indicated by arrowheads, double arrowheads and an asterisk, respectively.

could not be phosphorylated by c-Src, this 65-kDa protein binding was due to tyrosine phosphorylation in the cytoplasmic domain of PECAM-1.

When c-Src phosphorylated GST-PECAM-1Cyt was used as an affinity ligand, a polypeptide with the same molecular weight was recovered from the BAEC lysate (Fig. 1B). Binding of this 65-kDa polypeptide to the affinity matrix was dependent on the tyrosine phosphorylation. Affinity matrices made by GST (data not shown), non-phosphorylated GST-PECAM-1Cyt or GST-PECAM-1CytΔEx14, which lacked 18 amino acids including the whole consensus tyrosine phosphorylation motif [6], could not retain the 65-kDa polypeptide (Fig. 1C).

A number of protein kinases have been known to have an autophosphorylation activity. Whether or not the 65-kDa protein possessed this activity was tested by an in vitro autophosphorylation assay of the material bound to the affinity matrices (Fig. 1C). Although the 65-kDa band was phosphorylated, judging from the amount of proteins in the SDS-PAGE gel (see Fig. 1B), the phosphorylation level was roughly comparable to that of GST-PECAM-1Cyt. This result suggests that the 65-kDa protein is not a kinase. However, the material bound to the tyrosine phosphorylated PECAM-1 cytoplasmic domain actually contained certain kinase activities including one which effectively phosphorylated the PECAM-1 cytoplasmic domain. In contrast to the 65-kDa and the GST-PECAM-1Cyt bands, two higher molecular weight phosphorylated bands showed much higher relative levels of phosphorylation, suggesting that these two polypeptides were the candidates for putative PECAM-1 kinases. Small amounts of these two high molecular weight bands were also found in the material bound to the non-phosphorylated PECAM-1 cytoplasmic domain.

### 3.2. *The 65-kDa protein is SH-PTP2*

We purified a total of 5 μg of the 65-kDa polypeptide from $2\times10^9$ BAECs using the affinity matrix, obtained peptide fragments by 'in-gel' tryptic digestion, and then sequenced 7 fragments. Obtained sequences were W6FHPNITGVEAENLLLTR (the number indicated the corresponding amino acid number of the human SH-PTP2 sequence [13], completely matched with the human sequence), N58TGDYY (complete match), W112FHGHLSGK (complete match), G133SFLVR (complete match), S140QSLPGDFVLSVR (one aa change from H to L), T414VWQYHFR (complete match) and V579YENVGLMQQQK (complete match). All except one were identical to different parts of the human SH-PTP2 sequence and the fragment that did not show perfect identity contained one substitution out of 13 residues. These fragments did not show such a level of homology with any other known sequences including SH-PTP1. This result strongly suggests that the 65-kDa protein is a bovine homologue of SH-PTP2.

### 3.3. *Tyrosine phosphorylated PECAM-1 is a major SH-PTP2 binding protein in BAECs*

The association of PECAM-1 with SH-PTP2 was also observed in living BAECs. By immunoprecipitation, PECAM-1 was recovered as 128 and 110-kDa bands from BAECs. The 110-kDa molecule most likely represented a PECAM-1 form

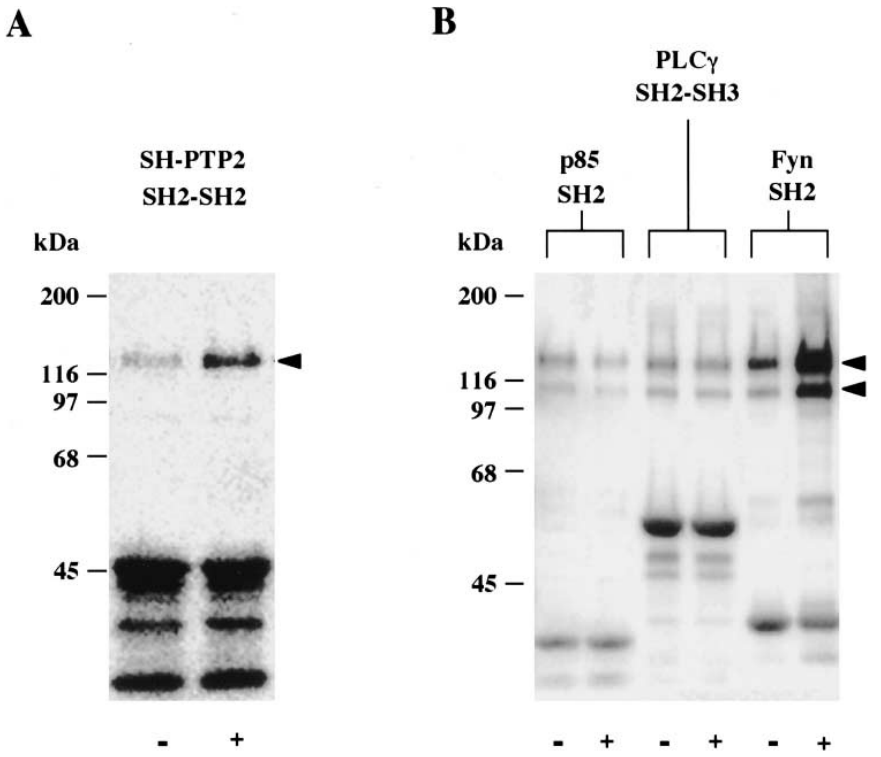


Fig. 3. SH2 domain dependent association between PECAM-1 and SH-PTP2 or Fyn kinase. The lysate from non-stimulated control cells (−) or osmotically stimulated cells (+) was applied onto affinity columns each containing 3 μg of various SH2 domains expressed as GST fusion proteins. The bound PECAM-1 (arrowhead) was detected by anti-PECAM-1 immunoblots. The stimulation-dependent increase in the bound PECAM-1 level is clear when GST fusion proteins containing two SH2 domains of SH-PTP2 (A) or the Fyn kinase SH2 domain (B, right lanes) are used as the affinity ligands, while no significant binding is detected when the SH2 domain of PI3 kinase p85 subunit (B, left lanes) or the SH2-SH2-SH3 domain of PLCγl (B, central lanes) is used. The amount of the 110-kDa PECAM-1 band varies among different cultures of BAECs [4,6].

with a lesser degree of glycosylation [6]. About 3-fold increase in the PECAM-1 tyrosine phosphorylation level was observed in ECs exposed to the hyperosmotic medium for 3 min (Fig. 2A). Immunoprecipitates with anti-SH-PTP2 from hyperosmotically stimulated BAECs contained an increased amount of PECAM-1 than that from the non-stimulated cells (Fig. 2B). Reciprocally, anti-PECAM-1 immunoprecipitates showed a stimulus-dependent increase in the coimmunoprecipitated SH-PTP2 (Fig. 2C). When anti-SH-PTP2 immunoprecipitates were probed with anti-phosphotyrosine, four major bands, about 170, 128, 105-120 and 40 kDa, were apparent (Fig. 2A). Although the 110-kDa PECAM-1 band overlapped with the 105–120-kDa band, the 128-kDa PECAM-1 band was clearly detected. SH-PTP2 itself also showed an increased level of tyrosine phosphorylation in the stimulated cells (Fig. 2A). These data demonstrate that tyrosine phosphorylation dependent interaction between PECAM-1 and SH-PTP2 does take place in BAECs and that PECAM-1 is a major SH-PTP2 binding protein in these cells.

### *3.4. Tyrosine phosphorylated PECAM-1 interacts with SH2 domains*

The association of tyrosine phosphorylated PECAM-1 and SH-PTP2 may be mediated by binding between SH2 domains of SH-PTP2 and phosphotyrosine residue(s) of PECAM-1. To prove this, we examined the binding capacity of PECAM-1 to various SH2 domains expressed as GST fusion proteins. PECAM-1 from the hyperosmotically stimulated BAECs showed increased binding to the SH-PTP2 (Fig. 3A) and the Fyn kinase SH2 domains (Fig. 3B) than that from control cells. PECAM-1 did not effectively bind to the PI3 kinase p85 SH2 domain or the PLCγ SH2-SH2-SH3 domain (Fig. 3B). GST alone or the Fyn kinase SH3 domain did not bind to PECAM-1 (data not shown). Our data indicate that tyrosine phosphorylated PECAM-1 provides binding site(s) for SH2 domains in a sequence specific manner as reported for various SH2 domain binding motifs [23].

### *3.5. SH-PTP2 translocates to cell-cell adhesion sites when ECs are osmotically stimulated*

PECAM-1 is known to be localized to cell-cell adhesion sites in ECs [8,9]. This localization pattern did not significantly change when cells were mechanically stimulated (Fig. 4A, B), at least during 5 min of the stimulation. SH-PTP2 is thought to be principally a cytoplasmic soluble protein [10] and we have found diffuse cytoplasmic staining with anti-SH-PTP2 in non-stimulated BAECs (Fig. 4C). When BAECs were stimulated by hyperosmotic condition, anti-SH-PTP2 staining appeared at cell-cell adhesion sites (Fig. 4D), suggesting that SH-PTP2 translocated and anchored to cell-cell adhesion sites, presumably, by the association with tyrosine phosphorylated PECAM-1.

## 4. Discussion

In this study we have shown that tyrosine phosphorylated

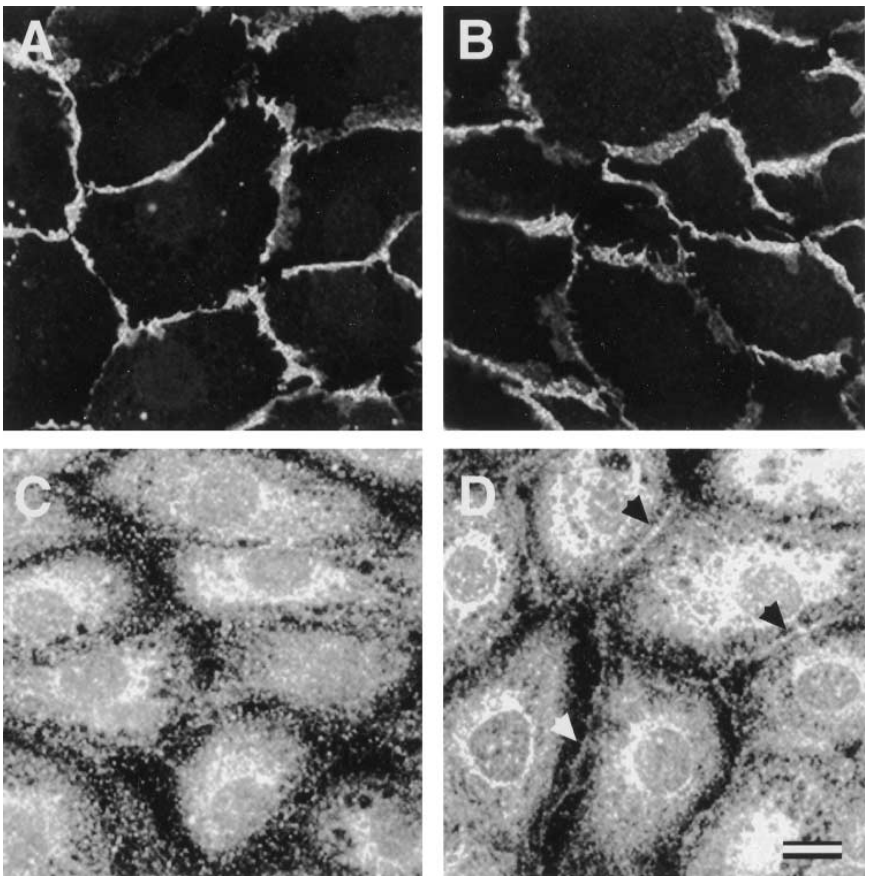


Fig. 4. Stimulation dependent translocation of SH-PTP2 into cell-cell adhesion sites. Confocal immunofluorescence micrographs showing BAECs stained with anti-PECAM-1 (A and B, a single optical section at the cell-cell adhesion level) and with anti-SH-PTP2 (C and D, an extended focus image reconstructed from 11 optical sections separated by 0.6 μm intervals). PECAM-1 is distributed at cell-cell adhesion sites and this localization appears unchanged between non-stimulated control BAECs (A) and cells exposed to the hyperosmotic condition for 3 min (B). Although a diffuse cytoplasmic staining pattern of SH-PTP2 is principally unchanged by the osmotic stimulation, SH-PTP2 becomes localized at cell-cell adhesion sites in the stimulated cells (D, arrowheads). This localization is hardly seen in non-stimulated control cells (C). Scale bar, 10 μm.

PECAM-1 is a major protein that associates with SH-PTP2 in BAECs. Our results indicate a direct binding of the two molecules mediated by the SH-PTP2 SH2 domains. The cytoplasmic domain of bovine PECAM-1 contains six tyrosine residues and five of them are conserved among human ([7], M28526), bovine ([6], D82082), porcine (X98505) and murine ([24], L06039) PECAM-1 forms. Two of them (Tyr690 and Tyr713 in the bovine form) are in a pYXX(L/V/I) sequence motif which is known as the consensus SH-PTP2 SH2 binding motif [23]. SH-PTP2 has two SH2 domains and the engagement of both domains results in massive activation of its phosphatase activity [25] which may be essential for SH-PTP2 mediated signal transduction [14,15,17]. Recently, SHP substrate 1 (SHPS-1) was purified as a major SH-PTP2 binding protein from v-Src transformed rat fibroblasts [26]. SHPS-1, also reported as BIT (a brain immunoglobulin-like molecule with tyrosine-based activation motifs) [27], is a membrane glycoprotein belonging to the immunoglobulin super family and has two pairs of tyrosine residues, Y408ADL-a.a.$_{20}$-Y432ASI and Y449ADL-a.a.$_{20}$-Y473ASI, in the cytoplasmic domain. Ohnishi et al. [27] demonstrated that each of the two sequences acted as a motif for simultaneous binding of the two SH-PTP2 SH2 domains. The PECAM-1 sequence, Y690TEV-a.a.$_{19}$-Y713SEV, shows homology to the two sequences of SHPS-1, suggesting that PECAM-1 also provides a bivalent binding site for SH-PTP2. Detailed binding analyses of phosphotyrosine containing peptides to the N-terminal SH2 domain of SH-PTP2 have shown that a group of peptides having (D/E/N/Q)pY(I/V/T)(D/E/N/Q)(L/V/I)(D/E/N/Q)(hydrophobic residue) motifs represents the strongest binding site for the SH2 domain [28]. The PECAM-1 cytoplasmic domain of all the known species contains a completely matched motif sequence at around Y690, suggesting that pY690 binds to the N-terminal SH2 domain of SH-PTP2.

PECAM-1 could not bind to the PLCγ or the PI3-kinase SH2 domain but did to the Fyn kinase SH2 domain in a tyrosine phosphorylation dependent manner. The result is consistent with the sequence feature of the PECAM-1 cytoplasmic domain: there is no tyrosine residue in the consensus motif for the PLCγ or the PI3-kinase SH2 domains [23], while both Y690TEV and Y713SEV of PECAM-1 fulfill the sequence requirement for the binding to Src family kinase SH2 domains: an acidic residue at the pY+2 and a hydrophobic one at the pY+3 position [23]. We have already shown that c-Src phosphorylates and binds to the PECAM-1 cytoplasmic domain in vitro [6]. The present result suggests that

this binding is mediated by the Src SH2 domain and that at least one of the PECAM-1 motifs has a dual specificity for SH2 binding.

In ECs, PECAM-1 is localized at cell-cell adhesion sites and participates in inter-endothelial cell adhesion by homophilic and heterophilic binding mechanisms [8–10]. The cell-cell adhesion site is also a cytoskeleton-plasma membrane attachment site and has been proposed to be one of the possible locations where putative mechanosensing machineries are present [3,6]. Interestingly, the PECAM-1 tyrosine phosphorylation in mechanically stimulated ECs was inhibited in cells pretreated by an actin filament disrupting agent cytochalasin D but was potentiated by phalloidin pretreatment, an actin filament stabilizer (unpublished observation). These observations indicate cytoskeletal involvement in the PECAM-1 phosphorylation. Our data suggest that PECAM-1 is a component of a mechanosensing machinery in ECs acting upstream of SH-PTP2.

When ECs are mechanically stimulated, such as fluid flow and osmotic shock, PECAM-1 is tyrosine phosphorylated and a portion of SH-PTP2 shifts its localization to the plasma membrane. We suggest that tyrosine phosphorylated PECAM-1 on the plasm membrane provides anchoring sites for SH-PTP2 and possibly for Src family kinases. Significance of these molecular association in the mechanical stimulus-induced signal transduction in ECs has not yet been elucidated. However, two possible roles can be suggested. One is activation of the MAP kinase cascade through the activation of SH-PTP2. The other is activation of Src family kinases by SH-PTP2-dependent dephosphorylation of their inhibitory C-terminal phosphotyrosine residue (Y527 in c-Src) [29] and by competitive participation of the PECAM-1 SH2 binding motif(s) in the inhibitory intramolecular binding between the Src SH2 domain and the C-terminal phosphotyrosine residue [30]. Recent findings have demonstrated that ERK and JNK/SAPK are activated in ECs stimulated by fluid shear stress [18–21]. Requirement for tyrosine kinase activity [20,21] and dependency of Ras mediated signaling [21] were reported in these MAP kinase activation pathways. Our preliminary data showed that both p42 ERK and c-Src were also activated in hyperosmotically treated BAECs. We suggest that PECAM-1 tyrosine phosphorylation mediated signaling participates, at least partly, in mechanical stimuli-induced MAP kinase activation in ECs.

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