Phosphorylation of a 72-kDa protein in PDGF-stimulated cells which forms complex with c-Crk, c-Fyn and Eps15

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Abstract Ligand-induced activation of the β-receptor for platelet-derived growth factor (PDGF) induces tyrosine phosphorylation of a number of downstream signaling proteins. In the present study, we used two-dimensional gel electrophoresis to characterize the spectrum of proteins phosphorylated in response to PDGF stimulation in porcine aortic endothelial cells expressing PDGF β-receptors. Several previously known substrates for the PDGF β-receptor were identified as well as a novel substrate of 72 kDa. The 72-kDa component could be co-immunoprecipitated in complex with the adaptor protein c-Crk, the non-receptor tyrosine kinase c-Fyn and the signaling molecule Eps15. The results obtained suggests that the 72-kDa protein might play an important role in signaling via the PDGF β-receptor, coupling non-receptor tyrosine kinases of the Src family with c-Crk and Eps15.

Key words: Platelet-derived growth factor; Receptor; c-Crk; c-Fyn; Eps15

1. Introduction

Platelet-derived growth factor (PDGF) is a mitogen and a chemoattractant for connective tissue and smooth muscle cells. It is a dimeric molecule consisting of disulfide-bonded A- and B-polypeptide chains, which form three different isoforms, PDGF-AA, -AB or -BB (reviewed by [1,2]). The different forms of PDGF bind with different affinities to two structurally related protein tyrosine kinase receptors; the α-receptor binds both the A- and B-chains with high affinity, while the β-receptor only binds the B-chain.

Ligand binding induces dimerization of the PDGF receptors, leading to activation of the intrinsic tyrosine kinase activity and trans-phosphorylation of tyrosine residues in the dimeric receptor complex. The phosphorylated tyrosine residues in the intracellular part of the receptors then function as docking sites for signaling molecules containing Src homology 2 (SH2) domains [3], thereby bringing downstream effector molecules in close contact with the receptor tyrosine kinase. For the PDGF β-receptor, more than 10 autophosphorylation sites have been identified (reviewed by [4]) as well as one site which becomes tyrosine-phosphorylated by Src kinases [5]. Sequences C-terminal of the phosphorylated tyrosine residues determine the specificity and affinity of the SH2 domain interactions [3]. A number of different signal transduction molecules have been shown to bind to individual phosphorylated tyrosine residues, including members of the Src family of non-receptor tyrosine kinases, the p85α subunit of the PI3′ kinase, the Ras GTPase-activating protein (GAP), the tyrosine phosphatase SHP2, members of the signal transducers and activators of transcription (STAT) family, phospholipase C-γ and the adaptor molecules Grb2, Shc and Nck [4]. Thus, a number of distinct signal transduction pathways are initiated at the activated PDGF β-receptor which lead to different biological responses, such as chemotaxis, mitogenicity and differentiation.

In order to characterize signal transduction via the PDGF β-receptor, we have studied protein phosphorylation in PDGF-BB-stimulated cells labeled with [^{32}P]orthophosphate, followed by analysis by high-resolution two-dimensional (2D) polyacrylamide gel electrophoresis.

2. Materials and methods

2.1. Materials

[^{32}P]Orthophosphate (40 mCi/ml), [^{35}S]methionine and [^{35}S]cysteine (Promix) were obtained from Amersham (UK) and leupeptin, phenylmethylsulfonyl fluoride (PMSF) and DNaseI (grade II) and RNaseA from Boehringer Mannheim (Mannheim, Germany). Acrylamide, NN′-methylene bisacrylamide, sodium dodecyl sulfate (SDS) and carbamylated creatine kinase isoelectric point marker were obtained from BDH (Poole, UK) and Triton X-305, Nonidet P-40 (electrophoresis purity grade) and sodium ortho-vanadate from Sigma (St. Louis, USA). Urea (ultra pure grade) and PY20 antiphosphotyrosine monoclonal antibody were obtained from Schwarz/Mann Biotech (ICN, Cleveland) and 1G2 antiphosphotyrosine monoclonal antibody from Oncogene Science (Cambridge, MA). Affinity-purified rabbit antiphosphotyrosine antibodies were prepared as described [6]. Cellulose thin-layer chromatography plates were obtained from Merck (Darmstadt, Germany). Servalytes were purchased from Serva (Heidelberg, Germany) and Ampholytes 5-7 and 3.5-10 from Pharmacia (Uppsala, Sweden).

2.2. Labeling of cells with [^{32}P]orthophosphate

Confluent 75 cm^2 cultures of porcine aortic endothelial (PAE) cells expressing the PDGF β-receptor were serum-starved for 24 h in 0.3% fetal calf serum (FCS) and then labeled for 3 h at 37°C in phosphate-free Ham's F12 medium, supplemented with 0.1% dialyzed FCS, 20 mM Hepes pH 7.4 and containing 1 mCi/ml carrier-free [^{32}P]orthophosphate. Cells were then pre-incubated for 10 min with 100 μM sodium ortho-vanadate before ligand stimulation, in order to inhibit dephosphorylation of proteins by tyrosine phosphatases, and then incubated with or without PDGF-BB (100 ng/ml) for 7 min at 37°C. After washing twice in TBS (20 mM Tris pH 7.4, 150 mM NaCl, 200 μM sodium ortho-vanadate), cells were lysed in 1 ml lysis buffer which was either composed of 20 mM Tris pH 7.4, 1% (w/v) Nonidet P-40, 200 μM sodium ortho-vanadate, 50 mM NaF, 5 mM EDTA, 1% (v/v) Aprotinin (10 000 KIU/ml), 5 μg/ml leupeptin, 1 mM PMSF, 10% (v/v) glycerol), or the same buffer, supplemented with 0.1% (w/v) SDS and 0.5% (w/v) sodium deoxycholic acid (RIPA lysis buffer). Cells were left on ice for 10 min before lysates were centrifuged at 14 000×*g* for 10 min at 4°C. The supernatants were then incubated with 50 μl protein A-Sepharose beads end-over-end for 30 min, as a pre-clearing step. The cleared lysates then received

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50 μg each of RNaseA and DNaseI and were divided into aliquots for incubation with the appropriate antibodies for 2 h at 4°C. Protein A-Sepharose (50 μl) was then added and the incubation prolonged for an additional 45 min before immunoprecipitates were washed twice in lysis buffer, twice in lysis buffer containing 500 mM NaCl and finally once in lysis buffer again. The samples were eluted in 80 μl of buffer for isoelectric focusing (9.8 M urea, 2% (w/v) Nonidet P-40, 1% (w/v) Triton X-305, 2% (v/v) Ampholytes 7-9 (LKB) and 5% (v/v) β-mercaptoethanol) and then briefly heated to 60°C in a waterbath in order to elute the immunoprecipitated proteins; care was taken not to heat the samples in the presence of urea for more than 3 min to avoid carbamylation of proteins.

2.3. 2D polyacrylamide gel electrophoresis

Phosphorylated proteins were separated according to their isoelectric points in 18 cm long and 2 mm thick cylindrical polyacrylamide gels. The gel solution contained 10 g urea, 3.4 ml 10% (w/v) Nonidet P-40, 726.4 μl Servalytes pH 5–7, 181.6 μl Ampholytes 5–7, 1.36 ml Ampholytes 3.5-10.0, 2.42 ml double-glass-distilled water and 2.43 ml acrylamide/bisacrylamide solution (8.38% (w/v) acrylamide and 1.62% (w/v) bisacrylamide), which was sufficient for 40 gels. The procedure was performed essentially according to O'Farrell [13]. After electrophoresis for 18 h at 1200 V (0.1 mA/gel), the gels were equilibrated in 60 mM Tris pH 6.8, 2% (w/v) SDS, 5% (v/v) β-mercaptoethanol and 10% (v/v) glycerol for 10 min. Gels were then applied on top of 10% polyacrylamide gels (acrylamide : bisacrylamide, 30 : 0.15) for the second dimensional SDS-gel electrophoresis and sealed with agarose containing equilibration buffer (2.5 g agarose/250 ml). The gels were run in parallel in a Bio-Rad Protean II xi 2D Multi-cell system overnight at constant temperature (14°C). After electrophoresis, gels were either fixed in 10% (v/v) acetic acid, 40% (v/v) methanol for 2 h and dried before exposure on pre-flashed X-ray films or transferred to Immobilon-P membrane for phosphoamino-acid analysis. Alternatively, gels were fixed in 10% (v/v) glutaraldehyde for 90 min, washed in distilled water and incubated at 50°C, 1 M KOH for 3 h on a rotating platform, in order to hydrolyze serine-bound phosphate to more clearly visualize tyrosine-phosphorylated proteins. The gels treated with 1 M KOH were re-equilibrated in 10% acetic acid, 40% methanol, before drying.

2.4. Exposure of gels

In order to quantify the radioactivity in ^{32}P-labeled proteins, a Fuji BioImager and the Tina software was used. Gels were furthermore exposed at −70°C on pre-flashed Fuji RX X-ray films with intensifying screen.

2.5. Molecular mass and isoelectric point markers

Carbamylated creatine kinase (20 μg) was applied together with [^{35}S]methionine and [^{35}S]cysteine-labeled cell lysate (500 000 trichloroacetic acid-precipitable cpm in 3 μl, obtained from labeling of cells for 20 h in methionine- and cysteine-free MCDB 104 medium containing 10% FCS and 1 mCi/ml [^{35}S]methionine and [^{35}S]cysteine) on 2D gels. After silver staining, the gels were treated with Amplify (Amersham), dried and exposed on X-ray film. By alignment of the film with the silver-stained gel, pI values were assigned to each of the ^{35}S-labeled proteins according to the 38 different charge forms of carbamylated creatine kinase. By running ^{14}C-labeled molecular mass markers (Amersham) in the second dimensional SDS-gel electrophoresis together with the above-mentioned standards, approximate molecular masses could be assigned to the individual proteins.

2.6. Phosphoamino-acid analysis

Phosphoamino-acid analysis was performed according to Kamps and Sefton [14] by partial acidic hydrolysis of phosphoproteins in 6 M HCl at 110°C and separation on thin-layer cellulose chromatography plates together with phosphoamino-acid standards.

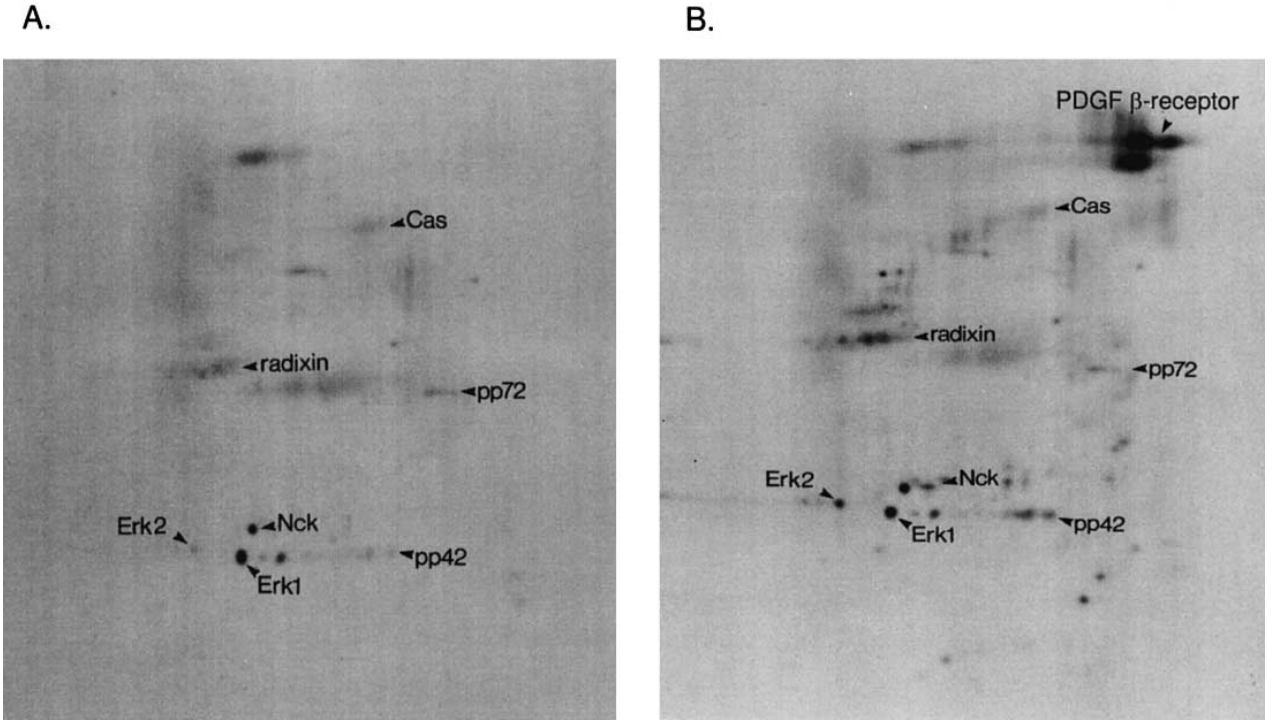

Fig. 1. Analysis by 2D electrophoresis of tyrosine phosphorylation in PDGF-stimulated cells. PAE cells expressing the PDGF β-receptor were labeled with [^{32}P]orthophosphate and stimulated with PDGF-BB for 7 min at 37°C (B) or left unstimulated (A). The cells were lysed in Nonidet P-40 lysis buffer and tyrosine phosphorylated proteins were immunoprecipitated by antiphosphotyrosine antibodies and separated by isoelectric focusing in the first dimension followed by SDS-gel electrophoresis in the second dimension. Phosphate bound to serine residues was hydrolyzed by incubation in 1 M KOH at 50°C and gels were then exposed to X-ray films. The position of the PDGF β-receptor as well as several other known signal transduction molecules, which were localized by immunoblotting with specific antibodies (data not shown), are indicated. The left and right sides of the gel are the basic (pH ≈7.0) and the acidic (pH ≈4.0) sides, respectively.

3. Results

3.1. Platelet-derived growth factor-induced phosphorylation of cellular proteins analyzed by high-resolution 2D gel electrophoresis

In order to identify proteins phosphorylated on tyrosine after stimulation of cells with PDGF, antibodies against phosphotyrosine were employed for immunoprecipitation. We found that a mixture of different anti-phosphotyrosine antibodies were more efficient than individual antibodies in precipitating tyrosine-phosphorylated proteins from a total cellular lysate (unpublished data). Therefore, we used a mixture of an affinity-purified rabbit polyclonal anti-phosphotyrosine antibody and two different monoclonal anti-phosphotyrosine antibodies (1G2 and PY20) to identify tyrosine-phosphorylated proteins in PDGF-stimulated cells. PAE cells expressing PDGF β-receptors were labeled with [^{32}P]orthophosphate and incubated in the absence or presence of PDGF-BB (100 ng/ml) for 7 min at 37°C, together with 100 μM sodium orthovanadate to inhibit protein tyrosine phosphatases. Cells were then washed and lysed in a Nonidet P-40 lysis buffer and subjected to immunoprecipitation with anti-phosphotyrosine antibodies. In order to reduce the background of ^{32}P radioactivity on the gels due to labeled RNA and DNA, RNaseA and DNaseI were included in the lysates during the immunoprecipitation. Moreover, we found that the unspecific background could be further reduced by including several high-salt washes (lysis buffer containing 500 mM NaCl) of the immunoprecipitates.

The immunoprecipitated proteins were eluted and applied to isoelectric focusing followed by SDS-gel electrophoresis. After electrophoresis in the second dimension, the gels were fixed and treated with 1 M KOH at 50°C for 2.5 h in order to hydrolyze serine-bound phosphate and thereby increase the sensitivity in the detection of tyrosine-phosphorylated proteins.

As seen in Fig. 1, stimulation of PDGF β-receptor expressing PAE cells with PDGF-BB led to an increase in phosphorylation of several proteins which were immunoprecipitated with anti-phosphotyrosine antibodies. The receptor itself was always the most heavily phosphorylated component. The identity of several of the other phosphorylated components was confirmed by immunoprecipitation with specific antisera, and included the structural protein radixin, and the adaptor protein Nck. In addition, unidentified 72- and 42-kDa components were observed (Table 1); the isoelectric points of these components were distinct from those of the similarly

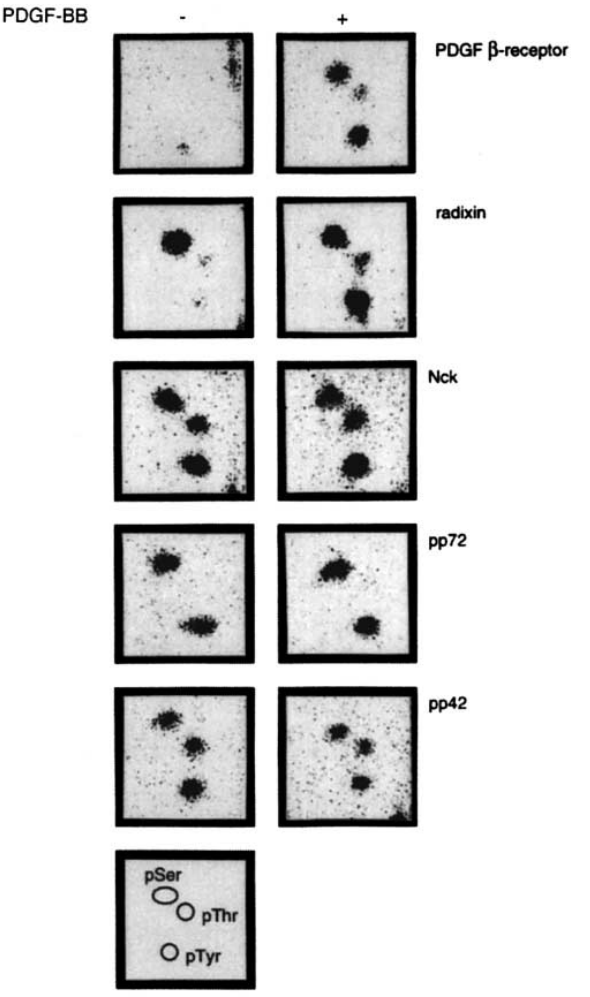

Fig. 2. Phosphoamino-acid analysis of components phosphorylated in response to PDGF-BB. Phosphoproteins immunoprecipitated with antiphosphotyrosine antibodies were separated by 2D electrophoresis as shown in Fig. 1 and transferred to an Immobilon-P membrane by electroblotting without prior treatment with 1 M KOH. After exposure of the membrane on a BioImager phosphorscreen, the phosphorylated proteins of interest were cut out and processed for phosphoamino-acid analysis by hydrolysis in 6 M HCl at 110°C. The phosphoamino acids were then separated by electrophoresis on thin layer chromatography cellulose plates; after staining with ninhydrin to visualize marker phosphoamino acids, plates were exposed on the BioImager phosphorscreen. The positions of the phosphoamino-acid standards have been indicated; pSer, phosphoserine; pThr, phosphothreonine; pTyr, phosphotyrosine.

Table 1
Tyrosine-phosphorylated components in PDGF-stimulated PAE cells expressing PDGF β-receptors

Phosphoprotein	Molecular mass	Isoelectric point	Phosphoamino-acid content	
			+PDGF-BB	Non-stimulated control
PDGF β-receptor	185000	5.4	**pTyr**, **pSer**, **pThr**	pTyr
Cas	130000	6.1	**pTyr**, **pSer**, pThr	pTyr, pSer, pThr
Radixin	80000	6.7	**pTyr**, **pSer**, **pThr**	pTyr, pSer, pThr
pp72	72000	4.5	**pTyr**, pSer	pTyr, pSer
Nck	47000	6.4	**pTyr**, pSer, pThr	pTyr, pSer, pThr
pp42	42000	5.9	**pTyr**, pSer, **pThr**	pTyr, pSer, pThr
Crk	39000	5.5	**pTyr**, **pSer**, **pThr**	pSer, pThr

Bold text indicates an increase in the content of this particular phosphoamino acid after stimulation with PDGF-BB.

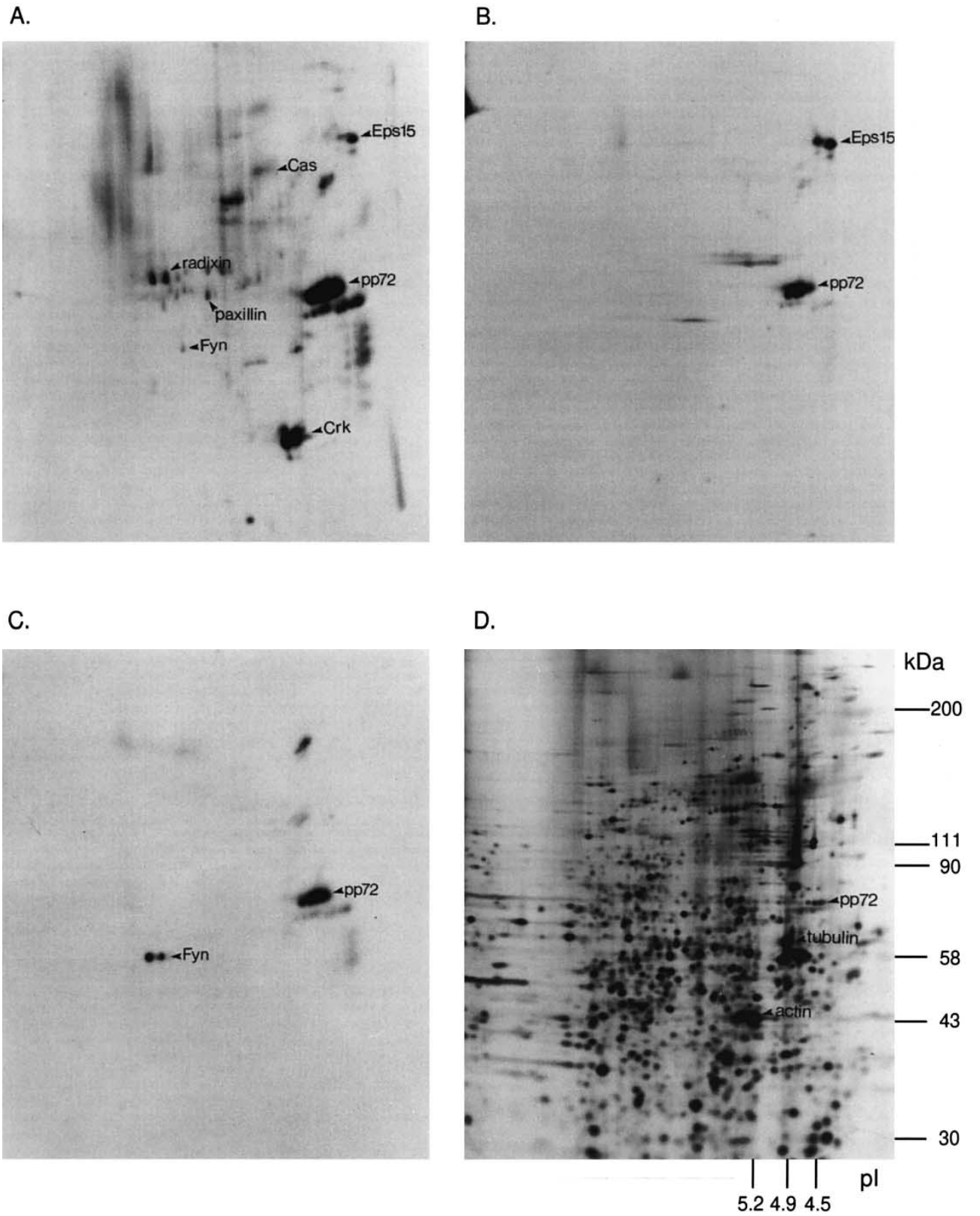

Fig. 3. Analysis by 2D gel electrophoresis of complex formation between the 72-kDa component, c-Crk, c-Fyn and Eps15 in PDGF-stimulated cells. Cells expressing the PDGF β-receptor were labeled with [^{32}P]orthophosphate and stimulated with PDGF-BB as described in Fig. 1 but lysed in RIPA lysis buffer containing SDS and sodium deoxycholic acid. The lysates were processed for immunoprecipitation with antibodies against c-Crk (A), Eps15 (B) or c-Fyn (C) and subjected to analysis by 2D gel electrophoresis. After electrophoresis in the second dimension, the gels were fixed and dried before exposure on pre-flashed X-ray films (only the exposures from the PDGF-BB-stimulated samples are shown). Proteins which have been identified by 2D gel electrophoresis and immunoblotting on total lysates of PAE cells are indicated as well as the 72-kDa protein. The gels are oriented with the basic side to the left and the acidic side to the right. The isoelectric point of the 72-kDa component was determined by comparison of ^{35}S-labeled protein with carbamylated creatine kinase PI standards (D).

sized SHP-2 and paxillin, and MAP kinase, respectively, as shown by separate immunoblotting experiments (data not shown).

The components phosphorylated in response to PDGF-BB (Fig. 1) were also subjected to phosphoamino-acid analysis. For this analysis, gels were not treated with 1 M KOH; after transfer to Immobilon-P membrane, individual components were cut out and subjected to phosphoamino-acid analysis (Fig. 2). The proteins phosphorylated after PDGF-BB stimulation in Fig. 1 were phosphorylated on tyrosine residues and most of them also on serine residues, while threonine phosphorylation was much less pronounced and in many cases undetectable (Table 1).

In the presence of sodium ortho-vanadate, we observed an increased tyrosine phosphorylation of many components and certain proteins were phosphorylated even without addition of growth factor, e.g. Nck and the unidentified 72- and 42-kDa components (Fig. 1). In contrast, the phosphorylation of the PDGF β-receptor and radixin were much less influenced by sodium ortho-vanadate (data not shown). This suggests that the dephosphorylation of tyrosine-phosphorylated components are differentially regulated by ortho-vanadate-sensitive tyrosine phosphatases.

3.2. *A 72-kDa phosphoprotein co-immunoprecipitates with c-Crk, c-Fyn and Eps15*

Several proteins which are known to become tyrosine-phosphorylated in response to PDGF-BB were not seen in the anti-phosphotyrosine immunoprecipitates, despite the fact that we detected the proteins by immunoblotting of cell lysates separated by 2D gel electrophoresis (data not shown). We therefore investigated the phosphorylation of such proteins in response to PDGF-BB stimulation by immunoprecipitation from ^{32}P-labeled cell lysates using substrate-specific antibodies. Using this method, several different phosphorylated molecules which were not detected by immunoprecipitation with the anti-phosphotyrosine antibodies (Fig. 1), including c-Crk, c-Fyn and Eps15, were seen. c-Crk is an adaptor molecule containing SH2 and SH3 domains [7] and c-Fyn is a tyrosine kinase of the Src family and also contains SH2 and SH3 domains [8]. In contrast, Eps15, which is phosphorylated after EGF stimulation of cells, lacks SH2 and SH3 domains [9].

During these investigations, we found that the 72-kDa phosphoprotein which was immunoprecipitated with anti-phosphotyrosine antibodies, occurred in complex with c-Crk, Eps15 and c-Fyn (Fig. 3A–C). The complexes resisted lysis in RIPA buffer which in addition to Nonidet-40 contains SDS and sodium deoxycholic acid, and washing in high-salt (0.5 M NaCl)-containing RIPA buffer, suggesting that the 72-kDa component interacted with c-Crk, c-Fyn and Eps15 with high affinity. Moreover, the co-immunoprecipitations of the 72-kDa phosphoprotein with c-Crk, c-Fyn and Eps15 were specific since antibodies against the adaptor protein Nck and the c-Crk-associated molecule Cas [10] were unable to bring down the 72-kDa phosphoprotein from the same cellular lysate (data not shown). As indicated in Fig. 3A, several phosphoproteins co-immunoprecipitate with c-Crk besides the dominating 72-kDa protein, including Cas, Fyn, radixin, paxillin and Eps15. Although neither c-Crk nor Nck could be co-immunoprecipitated with the Cas antibody, Cas could be co-immunoprecipitated by antibodies against both Crk and Nck (Fig. 3A, and data not shown). The 72-kDa phosphoprotein was not recognized in immunoblots using specific antibodies against c-Crk, c-Fyn or Eps15 (data not shown), excluding the possibility that any of these antibodies directly recognizes a common epitope in the 72-kDa phosphoprotein.

By co-migrational studies of total cellular proteins labeled with [^{35}S]methionine and [^{35}S]cysteine from PAE cells, and the 72-kDa ^{32}P-labeled phosphoprotein immunoprecipitated by antibodies against phosphotyrosine, c-Crk, c-Fyn or Eps15, we determined the isoelectric point of the 72-kDa protein to be 4.5 (Fig. 3D).

4. Discussion

In the present study, we have characterized the spectrum of proteins phosphorylated in response to PDGF-BB stimulation in porcine aortic endothelial cells expressing the human PDGF β-receptor. Interestingly, we identified an interaction between an unidentified 72-kDa phosphoprotein and c-Crk, c-Fyn and Eps15; whether all components can simultaneously be present in complex with the 72-kDa protein, remains to be determined. The phosphorylation of the 72-kDa protein was shown to increase both on serine and tyrosine residues in response to PDGF stimulation. The 72-kDa component was shown not to co-migrate with the similarly sized molecules SHP-2 and paxillin in 2D gel electrophoresis. It is also unlikely that the 72-kDa component is related to the tyrosine kinase Syk, which has a similar size, since the isoelectric points of the molecules differ (4.5 vs. 7.8).

The observation that Eps15 was co-immunoprecipitated with c-Crk by using c-Crk-specific antibodies confirms previous studies showing that the N-terminal SH3 domain of c-Crk binds specifically to a proline-rich sequence in domain III of Eps15 [11]. Crk has furthermore been shown to interact directly with the EGF receptor through a C-terminal phosphorylated tyrosine residue in the receptor and the SH2 domain of c-Crk [11], indicating that c-Crk might play an important role in docking Eps15 to tyrosine kinase receptors. Although Eps15 becomes tyrosine-phosphorylated in response to stimulation of the PDGF β-receptor (data not shown), it remains to be seen whether Eps15 can interact directly with the PDGF β-receptor, or whether c-Crk, by interaction with the PDGF receptor, could function as a docking protein as seems to be the case for the EGF receptor.

The expression of the transforming oncogene product v-Crk together with c-Src in 3Y1 cells, transforms cells much more efficiently then v-Crk alone [12]. When lysates from cells transfected with v-Crk on one hand, and with v-Crk combined with c-Src on the other, were analysed by immunoblotting with anti-phosphotyrosine antibodies, one major difference was observed; in lysates of cells co-transfected with v-Crk and c-Src there was a 70-kDa tyrosine-phosphorylated protein which was absent from cells transformed with v-Crk only [12]. Whether this 70-kDa protein corresponds to the 72-kDa phosphoprotein that we have shown to form complex with c-Crk in the present work, remains to be elucidated. The fact that the 72-kDa phosphoprotein in our studies could be co-immunoprecipitated with c-Fyn and Eps15, together with the increased tyrosine phosphorylation of the 72-kDa protein in response to PDGF-BB stimulation, suggest that the protein may take part in a signaling pathway involving complex formation of Src kinases with c-Crk and Eps15. Attempts are

now being made to purify this 72-kDa protein; future studies will focus on its identification and possible involvement in the signal transduction from the PDGF receptors.

Note added in proof: Using antiserum obtained from Dr Sugamura, we have recently found that the 72 kDa component described in the present communication is related to the adaptor molecule STAM recently identified by Takeshita et al., Biochim. BBiophys. Res. Commun. 225 (1996) 1035–1039.

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References

[1] C.-H. Heldin, B. Westermark, Cell Regul. 1 (1990) 555–566.
[2] Raines, E.W., Bowen-Pope, D.F. and Ross, R. (1990) in: Handbook of Experimental Pharmacology. Peptide Growth Factors and Their Receptors, Vol. 95, Part I (Sporn, M.B. and Roberts, A.B., Eds.), pp. 173–262, Springer, Heidelberg, Germany.
[3] T. Pawson, Nature (London) 373 (1995) 573–580.
[4] L. Claesson-Welsh, J. Biol. Chem. 269 (1994) 32023–32026.
[5] K. Hansen, M. Johnell, A. Siegbahn, C. Rorsman, U. Engström, C. Wernstedt, C.-H. Heldin, L. Rönnstrand, EMBO J. 15 (1996) 5299–5313.
[6] L. Rönnstrand, L. Terracio, L. Claesson-Welsh, C.-H. Heldin, K. Rubin, J. Biol. Chem. 263 (1988) 10429–10435.
[7] C.T. Reichman, B.J. Mayer, S. Keshav, H. Hanafusa, Cell Growth Diff. 3 (1992) 451–460.
[8] G. Superti-Furga, S. Fumagalli, M. Koegl, S.A. Courtneidge, G. Draetta, EMBO J. 12 (1993) 2625–2634.
[9] F. Fazioli, L. Minichielle, B. Matoskova, W.T. Wang, P.P. Di Fiore, Mol. Cell. Biol. 13 (1993) 5814–5828.
[10] R. Sakai, A. Iwamatsu, N. Hirano, S. Ogawa, T. Tanaka, J. Nishida, Y. Yazaki, H. Hirai, J. Biol. Chem. 269 (1994) 32740–32746.
[11] C. Schumacher, B.S. Knudsen, T. Ohuchi, P.P. Di Fiore, R.H. Glassman, H. Hanafusa, J. Biol. Chem. 270 (1995) 15341–15347.
[12] H. Sabe, M. Okada, H. Nakagawa, H. Hanafusa, Mol. Cell. Biol. 12 (1992) 4706–4713.
[13] P.H. O'Farrell, J. Biol. Chem. 250 (1975) 4007–4021.
[14] M.P. Kamps, B.M. Sefton, Anal. Biochem. 176 (1989) 22–27.