Dephosphorylation of autophosphorylated insulin and epidermal-growth-factor receptors by two major subtypes of protein-tyrosine-phosphatase from human placenta

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The identity of protein-tyrosine-phosphatases (PTPases) active against autophosphorylated insulin receptor was probed by using an insulin-receptor-related peptide phosphorylated on tyrosine (peptide 1142–1153). Two major peaks of PTPase activity were resolved from the particulate (Triton X-100-soluble) fraction of human placenta by chromatography on DEAE-cellulose. The two peaks were purified 1300–2300-fold; other peaks of PTPase activity (> 15%) were not detected. Properties of the PTPases indicated that they corresponded to subtypes 1A and 1B. Both subtypes appeared capable of catalysing dephosphorylation of all autophosphorylation sites in three domains of the insulin receptor, with no appreciable difference in the pattern of dephosphorylation detected by two-dimensional tryptic-peptide mapping. The tyrosine-1150 domain of the insulin receptor in triply phosphorylated form was found to be highly sensitive to the action of both PTPases, and was dephosphorylated at least 4 times faster than the doubly and singly phosphorylated forms of the tyrosine-1150 domain or phosphorylation sites in other domains by either PTPase. This is significant, as the level of the triphosphotyrosine-1150 species has been shown to correlate well with the capacity of the insulin-receptor tyrosine kinase to phosphorylate other proteins. Both subtypes also dephosphorylated autophosphorylated epidermal-growth-factor (EGF) receptor by > 95%. Placental particulate (and cytosolic) PTPase activity against either receptor distributed ~ 2:1 between subtypes 1A and 1B as assayed in the presence of EDTA. In summary, PTPases within two major subtypes have been identified as phosphotyrosyl-insulin and -EGF-receptor phosphatases *in-vitro*. The PTPases identified exhibit high affinities for substrates and high activities in cells, which is commensurate with the PTPases being important *in vivo* in controlling or reversing autophosphorylation-induced regulatory or signalling events.

INTRODUCTION

Insulin and epidermal-growth-factor (EGF) receptors are ligand-activated tyrosine-specific protein kinases (Ullrich & Schlessinger, 1990). Both receptors are transmembranous, with ligand-binding sites located extracellularly and tyrosine kinase active sites in cytoplasmic domains. Whereas the EGF receptor is a single polypeptide unit, the insulin receptor is composed of two types of subunit, linked predominantly in a β - α - α - β configuration by disulphide bonds. The α -subunit forms the major part of the insulin-binding site, whereas the β -subunit contains the tyrosine kinase active site. Mutagenesis of a critical lysine group involved in ATP binding in either receptor does not alter ligand-binding properties, but abrogates cellular responses to the ligand (Chen *et al.*, 1987; Chou *et al.*, 1987; Ebina *et al.*, 1987; Honegger *et al.*, 1987). Therefore the tyrosine kinase activities of the receptors appear essential for signal transduction.

Insulin and EGF receptors both undergo ligand-stimulated autophosphorylation on tyrosine residues, perhaps by a mechanism involving transphosphorylation. Four autophosphorylation sites (tyrosines 1068, 1086, 1148 and 1173) in the EGF receptor near the C-terminus of the receptor appear to define a region autoinhibitory to tyrosine kinase activity that can be relieved by autophosphorylation [reviewed by Carpenter & Cohen (1990) and Ullrich & Schlessinger (1990)]. There are at least seven sites of autophosphorylation in the insulin receptor: tyrosines 1316 and 1322 in the C-terminal domain; tyrosines 1146, 1150 and 1151 in the tyrosine-1150 domain; and putatively tyrosines 953 and 960 or 972 in the juxtamembrane domain (Tornqvist *et al.*, 1987, 1988; White *et al.*, 1988; Tavaré &

Denton, 1988; Tavaré *et al.*, 1988; King & Sale, 1990). Auto-phosphorylation of the insulin receptor activates by up to 200-fold the ability of the tyrosine kinase to phosphorylate other substrates (Rosen *et al.*, 1983; Yu & Czech, 1984). Consequently dephosphorylation, and not merely dissociation of bound insulin, is necessary to terminate tyrosine kinase activity.

Analysis of the state of autophosphorylation of the β -subunit during phosphorylation and the ability of the insulin receptor to catalyse substrate phosphorylation show that activation of the tyrosine kinase correlates best with phosphorylation of all three tyrosine residues (1146, 1150 and 1151) in the tyrosine-1150 domain (White *et al.*, 1988; Flores-Riveros *et al.*, 1989). The tyrosine-1150 domain in triply phosphorylated form is also, relative to other phosphorylated forms of the insulin receptor, highly sensitive to dephosphorylation by particulate and soluble protein-tyrosine-phosphatases (PTPases) present in rat liver extracts (King & Sale, 1990; Sale, 1991). The phosphatase-catalysed tri $\rightarrow$ di transition predominantly (80–85%) involved removal of phosphate from one of the twin phosphotyrosyl residues, 1150 or 1151. Disappearance of the triphosphotyrosine-1150 domain species catalysed by PTPases correlated well with deactivation of the tyrosine kinase (King *et al.*, 1991; Sale, 1991).

In this study we have set out to identify PTPases that can preferentially act on the triply phosphorylated tyrosine-1150 domain species and to address the question of whether a single PTPase or more than one PTPase is capable of dephosphorylating all the insulin-receptor autophosphorylation sites, or if different PTPases dephosphorylate different sites. Utilization of an insulin-receptor-related substrate in the isolation of PTPases from human placenta is described, and the action of the resolved PTPases

Abbreviations used: EGF, epidermal growth factor; PTPase, protein-tyrosine-phosphatase (EC 3.1.3.48); RCM, reduced, carboxyamidomethylated and maleylated.

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against autophosphorylated insulin receptor is characterized and compared with their action against autophosphorylated EGF receptor.

EXPERIMENTAL

Materials

DEAE-cellulose (DE52) and phosphocellulose paper were obtained from Whatman, Maidstone, Kent, U.K. Phosphocellulose (fine mesh), heparin-agarose and Brij 35 were from Sigma Chemical Co., Poole, Dorset, U.K. Other biochemicals and chemicals were obtained from sources described in King & Sale (1990) or Smith *et al.* (1988).

Purification of PTPases

All procedures were carried out at 4 °C. Preparation of particulate and soluble fractions and DEAE-cellulose chromatography were modified from Tonks *et al.* (1988a).

Preparation of particulate and soluble fractions from human placenta. Placentas were trimmed of amnion and chorion, cut into small pieces, washed in 10 mM-Tris/HCl, pH 7.4, containing 150 mM-NaCl, and homogenized for 3 × 15 s in a Waring Blender in 2 vol. of 10 mM-imidazole/HCl (pH 7.2)/0.25 M-sucrose/5 mM-EDTA / 0.1 % (v/v) β -mercaptoethanol, containing 0.002 % (w/v) phenylmethanesulphonyl fluoride, 1 mM-benzamidine and 1 μ g of leupeptin/ml. The homogenate was centrifuged at 27000 *g* for 1 h. The supernatant was filtered through glass wool and was designated the cytosolic fraction. The pellet was homogenized for 30 s in a Waring Blender in 2 vol. of the above imidazole buffer containing 0.5 % (v/v) Triton X-100, followed by stirring for 1 h and centrifugation at 27000 *g* for 1 h. After filtration through glass wool, the supernatant was designated the particulate (Triton X-100-soluble) fraction.

Chromatography on DEAE-cellulose. The cytosolic or particulate (Triton X-100-soluble) fractions from one placenta were stirred overnight with 30 ml of DEAE-cellulose slurry [equilibrated in Buffer A: 10 mM-imidazole/HCl (pH 7.2), 2 mM-EDTA, 0.1 % β -mercaptoethanol, 0.002 % phenylmethanesulphonyl fluoride and 1 mM-benzamidine] per g of protein. The slurry was then poured into a column (5 cm × ~15 cm), washed with Buffer A and eluted (250 ml/h) with a 1-litre linear 0–0.3 M-NaCl gradient in Buffer A. Pooled peaks of PTPase activity were dialysed overnight against Buffer A.

Chromatography on phosphocellulose. PTPases were passed three times through a phosphocellulose column (~1 ml bed volume/ml of dialysis residue) equilibrated in Buffer B [Buffer A containing 0.01 % (v/v) Brij 35]. The column was washed with 10 bed vol. of Buffer B and eluted (45 ml/h) with a linear 0–0.6 M-NaCl gradient (5 times the bed volume) in Buffer B. Pooled peaks of PTPase activity were dialysed overnight against Buffer B.

Chromatography on heparin-agarose. PTPases were recycled three times through a heparin-agarose column (10 ml bed volume) equilibrated in Buffer B. The column was washed with 5 bed vol. of Buffer B and eluted (45 ml/h) with a linear 0–0.6 M-NaCl gradient (50 ml) in Buffer B. After dialysis overnight against Buffer B, the pooled fractions were stored at –70 °C.

Preparation of 32 P-labelled substrates

Insulin and EGF receptors were co-purified from human placental membranes solubilized in Triton X-100 by affinity chromatography on wheat-germ-agglutinin-agarose and autophosphorylated (> 95 % of 32 P recovered in phosphoamino acids was attached to tyrosine) as previously described (King *et al.*, 1991). RCM (reduced, carboxamidomethylated and maleylated) lysozyme was prepared and phosphorylated by using the lectin-purified preparation of insulin and EGF receptors as

described by Tonks *et al.* (1988a). Peptide 1142–1153, Thr-Arg-Asp-Ile-Tyr-Glu-Thr-Asp-Tyr-Tyr-Arg-Lys, corresponding to amino acids 1142–1153 in the human insulin pro-receptor was synthesized by a solid-phase method (Merrifield, 1986). The peptide was phosphorylated by using the lectin-agarose-purified preparation of insulin and EGF receptors as previously described (King & Sale, 1988b). 32 P-peptide 1142–1153 was separated from non-peptide-bound 32 P by chromatography on an AG1-X2 (acetate form) column (5 ml) with 6 % (v/v) acetic acid, and freeze-dried. The stoichiometry of phosphorylation was approx. 0.1 mol/mol of peptide. Analysis by electrophoresis at pH 3.5 (pyridine/acetic acid/water, 1:10:89, by vol.) on cellulose thin-layer plates showed that under the phosphorylation conditions used the 32 P-peptide was ~90 % monophosphorylated. The 32 P-peptide was dissolved in 50 mM-Hepes (pH 7.4)/0.2 mM-dithiothreitol for use in dephosphorylation assays.

Dephosphorylation assays

32 P-labelled RCM lysozyme and 32 P-peptide 1142–1153. Samples (20 μ l) containing PTPase activity in 10 mM-imidazole/HCl (pH 7.2), 0.1 % β -mercaptoethanol, 0.002 % phenylmethanesulphonyl fluoride, 0.01 % Brij 35 and 1 mM-benzamidine, and samples (30 μ l) of 32 P-labelled RCM lysozyme (11 μ M, based on 32 P) or 32 P-labelled peptide 1142–1153 (10 μ l, 0.18 μ M) were mixed together after preincubation at 30 °C for 5 min. EDTA and poly(Glu,Tyr) (4:1) were present as indicated. After incubation for 20 min at 30 °C, 32 P released from the substrates was measured as described by Tonks *et al.* (1988a) or King & Sale (1988b).

Autophosphorylated receptors. 32 P-labelled receptors (~0.5 mg of protein/ml) were incubated with the indicated PTPase activity at 30 °C in 50 mM-Hepes (pH 7.4)/10 mM-EDTA containing 0.1 % Triton X-100 and 2 μ g of leupeptin/ml. Dephosphorylations were terminated by mixing 40 μ l portions with 10 μ l of SDS sample buffer, followed by SDS/PAGE and autoradiography (King & Sale, 1990). In some experiments insulin-receptor β -subunits were excised and subjected to two-dimensional tryptic-peptide mapping on cellulose thin-layer plates (King & Sale, 1990). Insulin-receptor amino acids are numbered in accordance with Ullrich *et al.* (1985).

RESULTS

A major objective of this study was the identification of PTPases active against autophosphorylated insulin receptor. To facilitate this, an insulin-receptor-related peptide was employed as substrate, rather than an artificial substrate. Artificial substrates may not contain the phosphotyrosine in the correct sequence, which could lead to critical species of PTPase being overlooked. A synthetic peptide (residues 1142–1153) derived from the regulatory (tyrosine-1150) autophosphorylation domain of the insulin receptor was chosen. Peptide 1142–1153, after phosphorylation on tyrosine, was a potent substrate for dephosphorylation by PTPases present in extracts of human placenta. PTPase activity was recovered in the particulate (Triton X-100-soluble) and cytosolic fractions of human placenta in the ratio ~1.5:1 with the 32 P-peptide as substrate.

Chromatography of the particulate (Triton X-100-soluble) fraction obtained from human placenta on DEAE-cellulose yielded two peaks of PTPase activity as assayed with 32 P-peptide 1142–1153, that were eluted at ~55 mM-NaCl and ~95 mM-NaCl (Fig. 1). With 32 P-labelled phosphotyrosyl RCM lysozyme as substrate, two peaks of PTPase activity were again resolved from the particulate Triton X-100-soluble fraction on DEAE-cellulose, eluted at ~55 mM- and ~95 mM-NaCl (Fig. 1) as found by Tonks *et al.* (1988a). These peaks were designated 1A

Fig. 1. Separation of PTPases from the particulate (Triton X-100-soluble) fraction of human placenta by chromatography on DEAE-cellulose

PTPase activity was assayed with ³²P-peptide 1142–1153 (●) or ³²P-RCM lysozyme (○) in the presence of 1.3 mM- or 5 mM-EDTA respectively. Results are from separate preparations; ~ 75 % of the PTPase activity applied to the columns was bound. The NaCl concentration was determined with a conductivity-meter.

and 1B respectively by Tonks *et al.* (1988a), who purified material from the peaks and showed that each predominantly [peak 1A ~ 85 % (designated 1A' by Tonks *et al.*, 1988a); peak 1B ~ 100 %] comprised a single species of PTPase. This was confirmed by the findings in the present work, using ³²P-peptide 1142–1153 as substrate, that purification of the particulate 1A or 1B DEAE-cellulose peaks on phosphocellulose (Figs. 2a and 2c) and heparin-agarose (Figs. 2b and 2d) yielded only one major peak of PTPase activity in each case. Phosphocellulose, in particular, has been shown to be highly effective in resolving

Table 1. Effect of poly(Glu,Tyr) (4:1) and EDTA on the activity of particulate PTPase subtypes 1A and 1B

Substrate	Condition	Activity (% of control)	
		1A	1B
³² P-RCM lysozyme	EDTA (10 mM)	110	136
³² P-RCM lysozyme	Poly(Glu,Tyr) (4:1) (5 μM)	109	62
³² P-peptide 1142–1153	Poly(Glu,Tyr) (4:1) (8.7 μM)	102	40

different species of PTPase (Jones *et al.*, 1989). The overall purification achieved in this study for the particulate 1A and 1B PTPase subtypes was ~ 2300- and ~ 1300-fold respectively. The yield of each subtype activity was ~ 40 % from the DEAE-cellulose step.

To confirm the distinct identity of the two particulate PTPases purified in the present work, the effects of known modulators of PTPase activity were tested (Tonks *et al.*, 1988b). Subtype 1B was found to be several-fold more sensitive to activation by EDTA and inhibition by poly(Glu,Tyr) (4:1) than the 1A subtype (Table 1). These observations, coupled with the elution position of the two species from DEAE-cellulose, confirm their identities (Tonks *et al.*, 1988a,b).

Dephosphorylation of autophosphorylated insulin and EGF receptors by PTPase subtypes 1A and 1B

Both autophosphorylated insulin and EGF receptors were dephosphorylated by either the particulate 1A or 1B PTPase subtype. The relative contribution of the two activities to receptor PTPase activity was estimated. For this purpose the PTPases

Fig. 2. Chromatography of particulate 1A and 1B PTPase subtypes on phosphocellulose and heparin-agarose

(a) PTPase subtype 1A, phosphocellulose. (b) PTPase subtype 1A, heparin-agarose. (c) PTPase subtype 1B, phosphocellulose. (d) PTPase subtype 1B, heparin-agarose. PTPase activity was assayed with ³²P-peptide 1142–1153 in the presence of 1.3 mM-EDTA.

Fig. 3. Determination of the distribution of particulate PTPase activity between subtypes 1A and 1B with autophosphorylated insulin and EGF receptors as substrate

PTPase subtypes 1A and 1B were separated by chromatography on DEAE-cellulose. PTPase activity distributed between subtypes 1A and 1B eluted from the DEAE-cellulose column in the ratio 1:2.3 with ^{32}P -peptide 1142–1153 as substrate at 10 mM-EDTA. Autophosphorylated insulin and EGF receptors were incubated for 15 min at 30 °C in the presence of 10 mM-EDTA with the indicated PTPase activities. PTPase activities were determined at 10 mM-EDTA with ^{32}P -peptide 1142–1153. Samples were subjected to SDS/PAGE, and the extent of dephosphorylation of the receptors was determined: $\square$, $\circ$, EGF receptor; $\blacksquare$, $\bullet$, insulin receptor; $\square$, $\blacksquare$, subtype 1A; $\circ$, $\bullet$, subtype 1B.

were separated only by DEAE-cellulose chromatography, to minimize the possibility of differential yields as far as practical. Autophosphorylated receptors were incubated with various amounts of the PTPase activities in the presence of 10 mM-EDTA. With either receptor ~ 6 times as much 1B activity was required to produce the same degree of dephosphorylation compared with the 1A activity in the experiment of Fig. 3, in which PTPase activity added was measured with ^{32}P -peptide 1142–1153 at 10 mM-EDTA. For example, 18.5 fmol/min per ml of subtype 1B and 2.6 fmol/min per ml of subtype 1A dephosphorylated the autophosphorylated insulin or EGF receptor to a comparable extent in 15 min. Standardizing PTPase activities with ^{32}P -RCM-lysozyme yielded similar results (not shown). In seven experiments, standardizing activities at 10 mM-EDTA with either substrate on four PTPase preparations, 4.6 ± 1.1 (mean $\pm$ S.E.M.) times as much 1B activity compared with 1A activity was required to produce equivalent dephosphorylation of either autophosphorylated insulin or EGF receptor. Particulate PTPase activity distributed between DEAE-cellulose peaks 1A and 1B in the ratio of 1:2.3 with ^{32}P -peptide or ^{32}P -RCM-lysozyme as substrate at 10 mM-EDTA. Thus twice as much particulate (Triton X-100-soluble) PTPase activity against autophosphorylated insulin or EGF receptor was attributable to the 1A subtype compared with the 1B subtype when measured at 10 mM-EDTA. A similar distribution was obtained for cytosolic human placental PTPase activity. In these experiments EDTA (10 mM) was employed to inhibit insulin-receptor tyrosine kinase activity. PTPase subtype 1B was activated relative to 1A by EDTA (Table 1; Tonks *et al.*, 1988b). Correction for the effect of EDTA gives a distribution of particulate placental PTPase activity against autophosphorylated insulin or EGF receptors between subtypes 1A and 1B of 2.5:1.

Dephosphorylation of insulin-receptor autophosphorylation sites by particulate 1A and 1B PTPase subtypes

Figs. 4 and 5 indicate that both PTPases were able to elicit dephosphorylation of all autophosphorylation sites present in

Fig. 4. Time course of dephosphorylation of insulin-receptor autophosphorylation sites by particulate PTPase subtype 1A, as analysed by two-dimensional tryptic-peptide mapping

(a) Tryptic phosphopeptides derived from the C-terminal domain: $\square$, doubly phosphorylated; $\triangle$, singly phosphorylated. (b) Tryptic phosphopeptides derived from the tyrosine-1150 domain; $\blacksquare$, triply phosphorylated; $\bullet$, doubly phosphorylated; $\blacktriangle$, singly phosphorylated. (c) Tryptic phosphopeptides derived from the putative juxtamembrane domain assuming doubly ($\circ$) and singly ($\blacktriangledown$) phosphorylated forms. The total yield of the phosphopeptides from the β -subunit was calculated from the ^{32}P in the β -subunit and the ratio of ^{32}P recovered in the phosphopeptides. The amount (fmol) of each phosphopeptide was then calculated by dividing the fmol of ^{32}P by the number of sites phosphorylated. Incubations contained equivalent PTPase activities against autophosphorylated insulin receptor (total sites, measured by determining the ^{32}P content of the β -subunit).

the C-terminal, regulatory (tyrosine-1150) and putative juxtamembrane domains of the β -subunit, as analysed by two-dimensional mapping of tryptic phosphopeptides. Although after 25 min incubation with the PTPases some of the singly phosphorylated forms of the tyrosine-1150 and putative juxtamembrane domains remained, the amounts present in incubations with either PTPase were less than the amount of the domains initially phosphorylated, indicating that some dephosphorylation of the singly phosphorylated species had occurred. The relative resistance of these singly phosphorylated species to dephosphorylation supports observations made with PTPases present in rat liver particulate and soluble extracts (King & Sale, 1990).

Fig. 5. Time course of dephosphorylation of insulin-receptor autophosphorylation sites by particulate PTPase subtype 1B as analysed by two-dimensional tryptic-peptide mapping

(a) Tryptic phosphopeptides derived from the C-terminal domain: □, doubly phosphorylated; △, singly phosphorylated. (b) Tryptic phosphopeptides derived from the tyrosine-1150 domain: ■, triply phosphorylated; ●, doubly phosphorylated; ▲, singly phosphorylated. (c) Tryptic phosphopeptides derived from the putative juxtamembrane domain assuming doubly (○) and singly (▼) phosphorylated forms. Other details are as described in the legend to Fig. 4. Incubations contained equivalent PTPase activities against autophosphorylated insulin receptor (total sites, measured by determining the ^{32}P content of the β subunit).

The tyrosine-1150 domain of the insulin receptor in triply phosphorylated form was highly sensitive to dephosphorylation by either PTPase (Figs. 4 and 5). Disappearance of this species was associated with increases in the doubly phosphorylated derivative and then the singly phosphorylated derivative as the diphospho-species disappeared. The doubly phosphorylated C-terminal and putative juxtamembrane domain species were dephosphorylated 4–7-fold more slowly than the triply phosphorylated tyrosine-1150 domain species by either PTPase. The singly phosphorylated derivative of the C-terminal domain was only weakly detected during dephosphorylation with either PTPase subtype. Consequently the singly phosphorylated C-terminal domain species is rapidly dephosphorylated once formed. This is analogous to the phosphorylation of this domain (Tornqvist *et al.*, 1987; White *et al.*, 1988; Tavaré & Denton, 1988), but in reverse. In summary, no significant differences in

the pattern of dephosphorylation of the various insulin-receptor autophosphorylation sites by particulate PTPase subtypes 1A and 1B was detected.

DISCUSSION

In this study a synthetic insulin-receptor-related peptide (residues 1142–1153) was primarily used in the identification of PTPases active against autophosphorylated insulin receptor. This was important because use of alien substrates in earlier studies could have led to critical species of PTPase, with the highest specificity, being overlooked or underestimated.

With phosphotyrosyl-peptide 1142–1153 as substrate, PTPase activity was detected in both the particulate (Triton X-100-soluble) and cytosolic fractions of human placenta in the ratio of ~1.5:1. This compares with a ratio (particulate:cytosolic) in rat liver of 4:1 with ^{32}P -peptide 1142–1153 as substrate and 2–3:1 with autophosphorylated insulin or EGF receptors as substrate (King & Sale, 1988a,b). However, the ratio in rat liver was measured for the whole particulate fraction, whereas the ratio in placenta applies to the Triton X-100-soluble component. Certain PTPases in the particulate fraction of human placenta that are believed to be associated with the cytoskeleton have been reported to be resistant to solubilization with Triton X-100 (Roome *et al.*, 1988). Because most PTPase activity against the autophosphorylated insulin receptor resided in the particulate fraction compared with the cytosolic fraction, greater emphasis has been given to characterizing particulate activities in the present study.

Two peaks of PTPase activity were purified from the particulate (Triton X-100 soluble) fraction of human placenta with ^{32}P -peptide 1142–1153 as substrate. On the basis of their properties, the species appear to correspond to the 1A and 1B subtypes characterized by Tonks *et al.* (1988b). These PTPases are believed to contain different catalytic subunits. Significantly, use of the ^{32}P -peptide 1142–1153 as substrate did not detect any other major species of PTPase activity during chromatography on DEAE-cellulose, phosphocellulose or heparin-agarose.

Of fundamental importance is whether a single species of PTPase can dephosphorylate all sites of autophosphorylation in the insulin receptor, or whether the concerted action of more than one PTPase is required. Also of importance is whether the same or different PTPases act on autophosphorylated insulin and EGF receptors. Both particulate PTPase subtypes 1A and 1B appeared able to dephosphorylate all autophosphorylation sites in the C-terminal, tyrosine-1150 and putative juxtamembrane domains of the insulin-receptor β -subunit. Both particulate subtypes 1A and 1B dephosphorylated autophosphorylated EGF receptor by >95%, and thus were not selective in the choice of receptor that could act as substrate. No appreciable differences in the pattern of dephosphorylation of the various autophosphorylation sites present in the insulin receptor by either the 1A or 1B particulate subtypes was detected (Figs. 4 and 5).

Autophosphorylation of the tyrosine-1150 domain of the insulin-receptor β -subunit appears important in controlling tyrosine kinase activity towards other substrates. Activation of substrate phosphorylation correlates well with production of the triphosphotyrosine-1150 domain species during phosphorylation (White *et al.*, 1988; Flores-Riveros *et al.*, 1989). The triphosphotyrosine-1150 domain and activation of substrate phosphorylation are both exquisitely sensitive to the action of rat liver PTPases present in particulate and soluble extracts, with disappearance of the triphospho species correlating well with deactivation of the tyrosine kinase (King *et al.*, 1991). Therefore it was important to identify specific species of PTPase that exhibit higher activity against the triphosphotyrosine-1150

domain species than against other phospho-insulin-receptor species. Results presented here show that in human placenta the two major particulate (Triton X-100-soluble) PTPase subtypes, 1A and 1B, possess such a property.

Dephosphorylation of insulin receptor autophosphorylated *in vitro* by the PTPase subtype 1B observed in the present work supports observations made by Tonks *et al.* (1988b) using purified insulin receptor. Microinjection of PTPase 1B into *Xenopus* oocytes has also been observed to block insulin-stimulated phosphorylation of tyrosine residues on a protein having a molecular mass in the same range as the β -subunits of insulin receptors or insulin-like-growth-factor 1 receptors (Cicirelli *et al.*, 1990). Autophosphorylated preparations of insulin and EGF receptors have also been reported to be substrates for the related high-molecular-mass putative receptor-linked PTPase, CD45, *in vitro* (Tonks *et al.*, 1990).

PTPase 1B has been considered to be the major type of PTPase present in human placenta, on the basis of studies with phosphotyrosyl RCM lysozyme (Tonks & Charbonneau, 1989). However, use of both phosphotyrosyl RCM lysozyme and phosphotyrosyl peptide 1142–1153 appears to underestimate the activity of PTPase subtype 1A, relative to activity against autophosphorylated insulin or EGF receptors (Fig. 3). PTPase subtype 1A accounted for up to 70 % of the particulate (Triton X-100-soluble) PTPase activity against autophosphorylated insulin or EGF receptors *in vitro* that was eluted from DEAE-cellulose, depending on the assay conditions. This proportion was applicable whether considering overall dephosphorylation of autophosphorylated insulin and EGF receptors or individual sites of the insulin receptor. Cytosolic placental PTPase activity distributed between subtypes 1A and 1B resolved on DEAE-cellulose in a similar ratio, with the autophosphorylated receptors as substrate. Thus PTPase subtype 1A has the potential to be at least as important as PTPase subtype 1B in controlling the function of insulin and EGF receptors, and it will clearly be important to address the respective roles of the two PTPases *in vivo*.

In summary, multiple PTPases have the capacity to dephosphorylate phosphotyrosyl-insulin receptor or -EGF receptor *in vitro*, including species within two major subtypes. Subtypes 1A and 1B together accounted for at least 75 % of the PTPase activity in the particulate (Triton X-100-soluble) and cytosolic fractions of human placenta with ^{32}P -peptide 1142–1153 as substrate. Both PTPase subtypes 1A and 1B have sub-micromolar K_m values for model protein substrates and high specific activities (Tonks *et al.*, 1988b). Thus both PTPases have the potential to modulate effectively the levels of phosphotyrosine in the receptors within the cell. Both subtypes also preferentially dephosphorylated the triply phosphorylated form of the tyrosine-1150 domain of the insulin receptor, which is consistent with observations in intact Fao or H_4 hepatoma cells at equilibrium, where there will be a delicate balance between kinase and phosphatase activities, and insulin receptor predominantly doubly phosphorylated in the tyrosine-1150 domain is observed (Tornqvist *et al.*, 1988; White *et al.*, 1988). Moreover, the activation state of the tyrosine kinase of the insulin receptor isolated from the insulin-stimulated Fao cells was low (White *et al.*, 1988). It follows that if the level of the triply phosphorylated

tyrosine-1150 domain species is controlled by PTPases, perturbing the system in favour of the insulin-receptor tyrosine kinase may be expected to increase the amount of the species. Indeed, in CHO.T and NIH 3T3 HIR 3.5 cells, in which the insulin-receptor concentration is increased by a factor of ~ 10 –100 by over-expression, significantly more triply phosphorylated tyrosine-1150 domain species is observed (Tavaré *et al.*, 1988). Thus control of the activation state of the insulin receptor by PTPases is likely to be important *in vivo*.

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