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Isolation and Characterization of a Tyrosyl Phosphatase Activator from Rabbit Skeletal Muscle and *Xenopus laevis* Oocytes†

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ABSTRACT: PTPA, a specific phosphotyrosyl phosphatase activator of the PCS_{H2} and PCS_L protein phosphatases, was purified up to apparent homogeneity from *Xenopus laevis* ovaries and rabbit skeletal muscle and highly purified from dog liver. PTPA appears as a 40-kDa protein in gel filtration, as well as in sucrose gradient centrifugation, and as a 37-39-kDa protein doublet in SDS-PAGE. Its estimated cellular concentration of 0.75 μM in oocytes or 0.25 μM in rabbit skeletal muscle is suggestive of an important role in the regulation of the cellular PTPase activity. The PTPase activation reaction of the PCS_L phosphatase is time-dependent, ATP and Mg^{2+} being essential cofactors [A_{50}(ATP) = 0.12 mM in the presence of 5 mM $MgCl_2$]. With RCM lysozyme as substrate, the specific activity of the PTPA-activated PCS_L phosphatase is 700 nmol of P_i/(min·mg). The pH optimum of the PTPase shifts from 8.5-9 in basal conditions to a neutral pH (7-7.5), and the A_{50} for the essential metal ion Mg^{2+} is decreased (3 mM). The activation is rapidly reversed in the presence of the substrate, and more slowly after removal of ATP·Mg. The PTPA-activated PCS_L phosphatase represents a major PTPase activity in the cytosol of *X. laevis* oocytes (at least 50% of the measurable PTPase with RCM lysozyme phosphorylated on tyrosyl residues). The PTPA activation is specific for the PTPase activity of the PCS_L and PCS_{H2} phosphatases, without affecting their phosphoseryl/threonyl phosphatase activity. However, effectors of the phosphorylase phosphatase activity, such as polycations and okadaic acid, also influence the PTPase activity. Phosphorylase *a* inhibits the activated PTPase activity (I_{50} = 5 μM). The PTPase activity of the other oligomeric PCS phosphatases (PCS_{H1} and PCS_M) is not influenced, suggesting an inhibitory role for some of their subunits. This activation is compared with the recently described PTPase stimulation of the PCS phosphatases by ATP/PP_i [Goris, J., Pallen, C. J., Parker, P. J., Hermann, J., Waterfield, M. D., & Merlevede, W. (1988) *Biochem. J. 256*, 1029-1034] and by tubulin [Jessus, C., Goris, J., Cayla, X., Hermann, J., Hendrix, P., Ozon, R., & Merlevede, W. (1989) *Eur. J. Biochem. 180*, 15-22].

A role for the phosphorylation of proteins on tyrosine residues in the control of cell proliferation is implied by the observation that protein tyrosine kinase activities are intrinsic to a number of growth factor receptors (Ushiro & Cohen, 1980; Kasuga et al., 1982; Petruzzelli et al., 1982; Ek et al., 1982; Jacobs et al., 1983) and that they are also subverted in the generation of a number of oncogenes (Downward et al., 1984; Sherr et al., 1985; Stern et al., 1986). The importance of these activities in signal transfer is evident from studies demonstrating that abolition of kinase activity through site-directed mutagenesis abolishes the signaling/transforming capabilities (Honegger et al., 1987; Chou et al., 1987; Chen et al., 1987). In the model systems that have been used to elucidate the control of cellular functions by phosphorylation, it has become increasingly clear that the role played by protein phosphatases is far from a passive one and that complex regulatory mechanisms exist to affect steady-state protein phosphorylation through alterations in protein phosphatase activity (Cohen, 1982; Merlevede et al., 1984; Ballou & Fischer, 1986; Goris et al., 1989a).

Evidence was presented by several laboratories for the existence of specific PTPases,[1] distinct from the mammalian acid

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[1] Abbreviations: PTPA, phosphotyrosyl phosphatase activator protein; PTPase, phosphotyrosyl phosphatase; *p*NPPase, *p*-nitrophenylphosphate phosphatase; PCS_{H1}, PCS_{H2}, PCS_M, PCS_L, and PCS_C phosphatases, polycation-stimulated, high (H), medium (M), and low (L) molecular weight phosphatases and the catalytic subunit of the same species; AMD phosphatase; ATP·Mg-dependent phosphatase; MAP_2, microtubule-associated protein; RCM lysozyme, reduced carboxamidomethylated and maleylated lysozyme; MLC, myosin light chains; kDa, kilodalton(s); SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; Bis-Tris, 2,2-bis(hydroxymethyl)-2,2',2''-nitrilotriethanol; TLCK, L-1-chloro-3-(4-tosylamino)-7-amino-2-heptanone hydrochloride; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride.

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Table I: Purification of Rabbit Muscle PTPA[a]

step	protein (mg)	activity (units)	specific activity (units/mg)	yield (%)
crude extract	14000			
45–80% ammonium sulfate precipitate	9700			
DEAE-Sepharose eluate	215	76000	353	100
Ultrogel AcA-34 eluate	142.7	42250	990	55.6
Q-Sepharose eluate	20.5	30000	1450	39.5
phenyl-Superose eluate	15.3	22500	15000	29.6
Mono-Q-1 eluate	4.45	16000	36000	21
Mono-P eluate	0.22	14500	65900	19
Mono-Q-2 eluate[b]	0.09	9000	100000	11.8

[a] Starting material was 300 g of fresh rabbit muscle. The activity of the DEAE-Sepharose eluate was taken as 100%. [b] Recovery of proteins and activity measured after concentration by extensive dialysis against 60% glycerol buffer.

Table II: Purification of Ovarian PTPA[a]

step	protein (mg)	activity (units)	spec. activity (units/mg)	yield (%)
tyrosine–agarose eluate	1715			
DEAE-Sepharose eluate	201.6	54000	267	100
Ultrogel AcA-34 eluate	77.5	40000	516	74
45–80% ammonium sulfate precipitate	42.3			
phenyl-Superose eluate	3.22	18000	5600	33
Mono-Q eluate	0.728	8600	12000	16
Mono-P eluate[b]	0.020	1900	95000	3.5

[a] Starting material was 67 g of fresh *Xenopus* ovary. [b] Recovery of protein and activity measured after concentration by extensive dialysis against 60% glycerol buffer.

and alkaline phosphatases as well as from the Ser/Thr phosphatases [Foulkes et al., 1983; Shriner & Brautigan, 1984; Brunati & Pinna, 1985; Okada et al., 1986; Tung & Reed, 1987; Tonks et al., 1988a,b; see Lau et al. (1989) for a review]. We have recently shown that the PTPase activity associated with the polycation-stimulated (PCS) phosphatases (Li, 1982; Chernoff et al., 1983; Foulkes et al., 1983) can be increased in several ways, so that they become quantitatively important PTPases. The phosphoseryl/threonyl phosphatase activity of the PCS phosphatases can be converted into a PTPase activity by an enzyme-directed effect of free ATP or PP_i (Hermann et al., 1988; Goris et al., 1988), and this PTPase can be influenced by the microtubular proteins tubulin and MAP2 (Jessus et al., 1989). Four distinct PCS protein phosphatases were isolated from skeletal muscle (Waelkens et al., 1987a). These enzymes are classified according to the molecular weight of the native enzymes as PCS_H (390-kDa), PCS_M (260-kDa), and PCS_L (200-kDa) phosphatases. They have the following basic subunit structure: PCS_{H1} ($2A_1$), (65/55/35)-kDa subunits; PCS_{H2}, (65/35)-kDa subunits; PCS_M, (72/65/35)-kDa subunits; and PCS_L ($2A_2$), (65/35)-kDa subunits, PCS_C being the 35-kDa catalytic subunit. They are further characterized by distinct regulatory properties and substrate specificities (Waelkens et al., 1987a,b). The PCS_{H2} phosphatase is derived from the PCS_{H1} enzyme by dissociation of the noncatalytic 55-kDa subunit but is clearly different from the PCS_L phosphatase: although they display a similar subunit structure, they have a different substrate specificity (Goris et al., 1986).

We now present evidence for a third mechanism that can regulate the PTPase activity of the PCS_L and PCS_{H2} phosphatases, involving a specific protein factor, the PTPase activator (PTPA), without affecting the phosphoseryl phosphatase activity. This protein factor was purified to apparent homogeneity from rabbit skeletal muscle and *Xenopus laevis* oocytes and was highly purified from dog liver. The mechanism of activation involving ATP and Mg^{2+} ions has been investigated, and the resulting PTPase activity compared with other PTPases both quantitatively and in its properties.

Experimental Procedures

The experimental procedures are presented as supplementary material. The mechanism of the PTPase activation of the PCS phosphatases by PTPA is not fully understood (see Results), even if it shows all the characteristics of an enzymatic reaction, the PCS phosphatase and ATP·Mg being substrates and PTPA a converting enzyme. Therefore, no direct evaluation of this conversion at the molecular level is possible, and only the change of the aryl phosphatase activity could be followed during the purification of PTPA. Since the activation is transient in the presence of the phosphotyrosyl substrate or *p*NPP, a short incubation time for the assay of the PTPase activity is mandatory. The routine assay of PTPA is based on the increased *p*NPPase activity of the PCS_L phosphatase (muscle or oocyte), under conditions where the increase in activity was directly proportional to the quantity of the factor added.

For the preparation of PTPase activator from rabbit skeletal muscle, a 45–80% ammonium sulfate precipitate of the low-speed supernatant was dialyzed and subjected consecutively to DEAE-Sephacel, Ultrogel AcA-34, Q-Sepharose chromatography, phenyl-Superose-, First Mono-Q, Mono-P, and second Mono-Q fast protein liquid chromatography (Table I). A very similar purification procedure was followed for the PTPA from *Xenopus* ovaries. However, the starting material was here the breakthrough of the Tyr–agarose column used in the purification of the PCS_L phosphatase from oocytes (Hermann et al., 1988). This purification is summarized in Table II.

Results

(1) Purification of PTPA. With a 45% $(NH_4)_2SO_4$ precipitation a large quantity of the endogenous *p*NPPase activity was removed, whereas the PTPA remained soluble and could be precipitated with 80% $(NH_4)_2SO_4$. The PTPA was therefore isolated from this fraction of rabbit skeletal muscle (Table I). During the purification the contaminating *p*NPPase could be completely removed, and absolutely no endogenous aryl phosphatase could be detected from the phenyl-Superose step onward. PTPA behaved as a single entity throughout the purification, with a yield of about 90 μg of protein from 300 g of muscle. This corresponds to a purification of 280-fold and a yield of 12% relative to step 3, the activity measurements being unreliable during the initial steps. However, on the basis

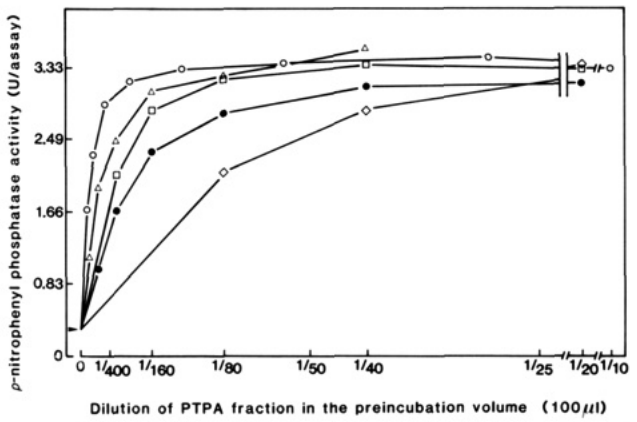

FIGURE 1: Assay of PTPA in the last five purification steps. Muscle PCS_L phosphatase was preincubated at 30 °C for 5 min with increasing concentrations of muscle PTPA of the different purification steps in the presence of 1.25 mM $MgCl_2$ and 0.25 mM ATP and assayed for *p*NPPase. (◇) Pool of the Q-Sepharose column (0.75 mg/mL); (△) pool of the phenyl-Superose column (4.4 mg/mL); (□) pool of the first Mono-Q column (0.89 mg/mL); (●) pool of the Mono-P column (60 µg/mL); (○) pool of the second Mono-Q column (150 µg/mL). The basal activity is indicated by the arrow.

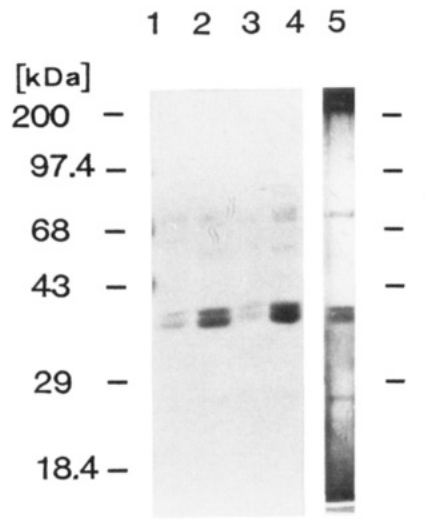

FIGURE 2: SDS–PAGE of purified PTPA from rabbit skeletal muscle and *X. laevis* oocytes. Ten-microliter aliquots of the three peak fractions (lanes 1–3) and of the pooled and concentrated peak (lane 4) of the second Mono-Q column of the muscle PTPA, and 10 µL of the peak fraction of the Mono-P column of the oocyte PTPA (lane 5), were subjected to electrophoresis. Proteins were stained with Coomassie Brilliant Blue (lanes 1–4) or silver stained (lane 5). The position of the molecular weight markers is indicated.

of the protein eliminated during these early stages (98%), and assuming a recovery of 50%, the specific activity in the crude extract can be estimated at 11 units/mg and the purification at 9100-fold. Assuming 50% H_2O/g of tissue, the cellular concentration of the PTPA can therefore be estimated at 250 nM.

The purification scheme for PTPA from *Xenopus* ovaries (Table II) is basically the same as for the purification from rabbit skeletal muscle, with the breakthrough of the tyrosine agarose column used in the purification of the PCS_L phosphatase from ovaries as starting material (Hermann et al., 1988). This procedure allows for the purification of PTPA and the PCS_L phosphatase from the same ovaries since it was known that the tyrosine agarose quantitatively removes the PCS_L phosphatase (Hermann et al., 1988). Making the same assumptions as for skeletal muscle, the concentration of PTPA in oocytes can be estimated at 750 nM. This can be compared to the 400 nM PCS_L phosphatase as can be calculated from the purification of this enzyme from *Xenopus* oocytes (Hermann et al., 1988), suggesting that the amount of PTPA cannot be the limiting factor in the activation of the PCS_L phosphatase in these cells. PTPA was also partially purified from isolated stage VI oocytes, prepared as described by Hermann et al. (1988), until and including the phenyl-Superose step. Quantitatively and qualitatively similar results were obtained, indicating that PTPA is present in the oocytes and apparently does not originate in substantial amounts from other tissues present in the ovaries such as follicle cells or connective tissue.

For the dog liver PTPA, the same purification procedure as for the skeletal muscle was used up until the chromatofocusing step, starting with the postribosomal supernatant as prepared in Goris et al. (1981). Very similar results were obtained, liver PTPA eluting at the same position in the different chromatograms (not illustrated).

The physical and enzymatic properties of PTPA purified from ovaries and from skeletal muscle were very similar including specific activity, molecular weight as determined by gel filtration, sucrose gradient centrifugation, and SDS–PAGE. The enzymatic properties were comparable, and no tissue specificity could be observed: PTPA from rabbit skeletal muscle could activate the oocyte PCS_L phosphatase while PTPA from oocyte could activate the muscle PCS_L phosphatase. The only difference observed (see further) was their different isoelectric point: oocyte PTPA was a little less acidic (p*I* = 5.8) than rabbit skeletal muscle or dog liver PTPA (p*I* = 5).

The purified PTPA had a specific activity of ±100 000 units/mg of protein. On SDS–PAGE, the muscle as well as the oocyte PTPA appeared as a protein doublet of 37–39 kDa (Figure 2). Activity is associated with this protein as could be demonstrated by electrophoresis under nondenaturing conditions and assay of the eluted proteins (not illustrated). It is not known whether both proteins are isomers or represent covalently modified forms. Both proteins show the same trypsin resistance and sensitivity to chymotrypsin (not shown). In native gels they migrate at the same position.

(*2*) *Physical Properties.* (*A*) *Molecular Weight.* The molecular weight of highly purified PTPA from skeletal muscle as well as from oocytes is 40 000 as determined by Superose 12 gel filtration or sucrose density gradient centrifugation. The same molecular weight is found early in the purification (step 4). Since a similar molecular weight is found in SDS–PAGE, it can be assumed that PTPA is a monomer with spherical dimensions.

(*B*) *Isoelectric Point and Stability.* On the basis of chromatofocusing, the isoelectric point of PTPA was estimated at ±5 for skeletal muscle and liver and ±5.8 for oocyte PTPA. At this low pH, PTPA was very unstable (see Experimental Procedures). PTPA is heat-labile, and its activity is completely lost upon heating at 95 °C during 5 min. PTPA is very resistant to trypsin treatment even at higher than equimolar concentrations of the protease, whereas chymotrypsin destroys its activity.

(*3*) *Interaction of PTPA with the PCS Phosphatases.* (*A*) *Activation.* Figure 3 shows the activation of different amounts of PCS_L phosphatase by increasing concentrations of purified PTPA. As can be seen, the final activity is clearly determined by the amount of the PCS_L phosphatase, whereas the activation can be saturated by increasing the concentration of PTPA. Within the limits of this experiment, the half-saturation point is independent of the concentration of the PCS_L phosphatase. With the purified PTPA, at the half-saturation

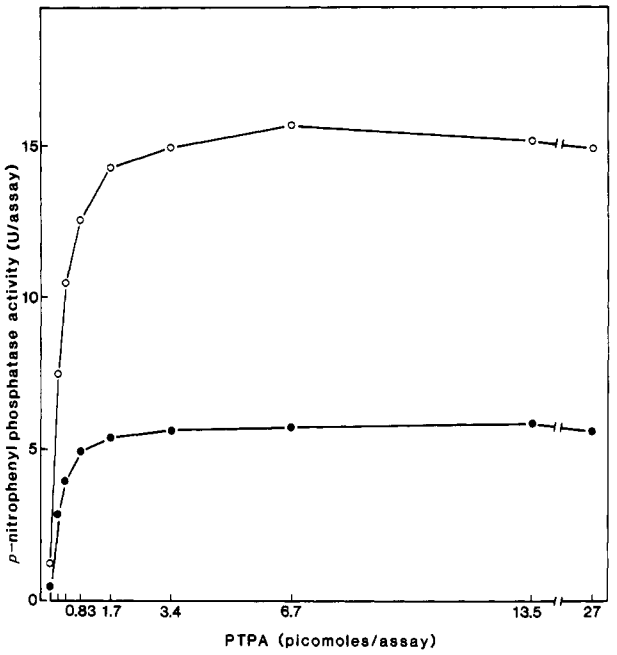

FIGURE 3: The *p*NPPase activity is determined by the amount of PCS_L phosphatase and can be saturated by increasing concentrations of PTPA. Two different amounts, 1.5 pmol (●) and 5 pmol (○) of muscle PCS_L phosphatase, were preincubated with increasing concentrations of muscle PTPA for 5 min at 30 °C in the presence of ATP·Mg and assayed for *p*NPPase activity.

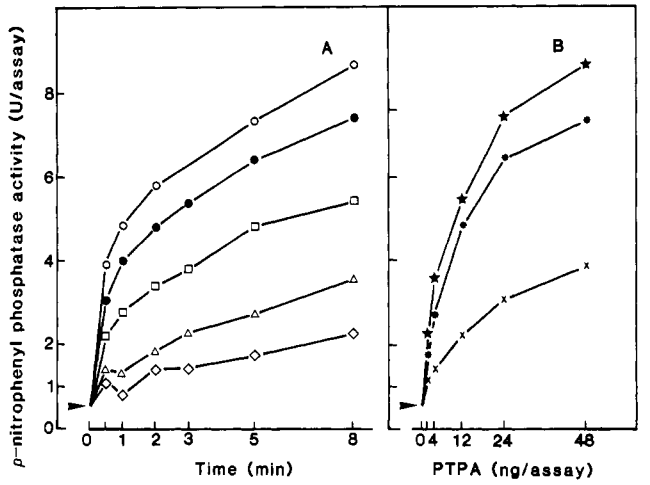

FIGURE 4: Time- and concentration-dependent activation of the *p*NPPase activity of PCS_L phosphatase by PTPA. (A) Muscle PCS_L phosphatase was preincubated at 30 °C for the indicated times in the presence of ATP·Mg and 48 ng (○), 24 ng (●), 12 ng (□), 3.6 ng (△), or 1.52 ng (◇) of muscle PTPA in a 100-μL preincubation mixture. *p*NPPase activity was assayed for 2.5 min at 30 °C. (B) The same data were plotted as a function of the PTPA concentration and activation times of 0.5 min (×), 5 min (*), and 8 min (★) by PTPA.

point the molar ratios PCS_L phosphatase/PTPA were respectively 6 and 20. As illustrated in Figure 1, the half-saturation point could be used to quantify PTPA at the different stages of purification.

The increase of the aryl phosphatase activity of the PCS_L phosphatase by PTPA is also dependent on the time these components are incubated in the presence of ATP·Mg (Figure 4). As illustrated, the initial velocity, as well as the final activity level reached, is dependent on the PTPA concentration used. The half-saturation point for PTPA (Figures 1 and 3) might therefore result from the combination of both parameters. Preincubation of different combinations, omitting one component, at 30 °C does not influence the time course of the activation.

Activation of the aryl phosphatase activity of the PCS_L phosphatase by PTPA is entirely dependent on, and highly specific for, the presence of ATP·Mg. The A_{50} for ATP was found to be 0.12 mM in the presence of 5 mM Mg^{2+}, and several other nucleotides and ATP analogues were not (ITP, ATPγS, AMPPNP, ADP, GDP, cyclic AMP, AMP, adenosine) or were much less effective (GTP, UTP). The pH optimum for the activation reaction was found to be rather broad (between 7 and 8). The phosphatase as well as the activator are less stable at pH values below 6.5 and above 9, so that this broad optimum probably reflects the stability of the proteins as well as the pH optimum of the reaction. A pH of 7.5 was adopted routinely for the activation reaction.

The requirement by PTPA of ATP·Mg as cofactor for the activation of the PCS_L phosphatase is very suggestive for a kinase reaction in the PTPase activation. Under conditions that ascertained an extensive activation of the aryl phosphatase activity of the PCS_L phosphatase, we used [γ-^{32}P]ATP or [^{35}S]ATPγS as possible phosphate donor and looked for the incorporation of phosphate into the proteins by SDS–PAGE and autoradiography. Purified PTPA (0.25 μg = 6.25 pmol) was incubated in 40 μL with various concentrations of PCS_L phosphatase (up to 750 nM) and [γ-^{32}P]ATP·Mg (1000–2000 cpm/pmol) for short (1-min) or longer (20-min) periods at 30 °C. In none of the conditions could a radiolabeled band of any significance be observed, even after a prolonged exposure time (up to 14 days), except for a very low phosphorylation of the two phosphatase bands (62 and 35 kDa), also observed in the controls without PTPA.

We tested also for the presence of the labile acyl phosphates (Martensen, 1984) by running gels in acidic conditions (Wuytack et al., 1984) and for the acid-labile histidine phosphate (Martensen, 1984) by taking precautions in the staining and destaining procedures. From all these results, and although ATP·Mg is an essential cofactor in the activation reaction, which shows the characteristics of an enzymatic reaction, PTPA seems not to be a Ser/Thr or Tyr kinase, nor is there formation of a stable phospho intermediate such as acyl phosphate or histidine phosphate. In addition, the effect of PTPA as a presumed kinase appears to be highly specific. None of the following potential kinase substrates were phosphorylated by PTPA as measured with the filter paper assay: histone H_1 (Sigma IIIS), histone IIA, casein α or β, phosvitine, protamine, inhibitor 1, phosphorylase, MLC, tubulin, or poly[Glu,Tyr (4:1)].

(*B*) *Deactivation.* When the *p*NPPase activity is monitored by following the OD at 410 nm continuously after the activation, it can be observed that the enzyme is rapidly deactivated as the ΔOD/min decreases with time ($t_{1/2}$ ±2–3 min). This is in contrast with the basal and the ATP-stimulated as well as the tubulin-stimulated *p*NPPase activity, which remains stable over a long period of time (Figure 5). A similar deactivation is observed when RCM lysozyme or MLC (not shown), both phosphorylated on tyrosyl residues, was used as substrate. These aryl phosphate substrates apparently even block the activation reaction. If PTPA and ATP·Mg are added in the phosphatase assay, without preincubation, no activation can be observed; if *p*NPP is added in the preincubation of the PCS_L phosphatase with PTPA and ATP·Mg, and the phosphatase activity is measured with tyrosyl-phosphorylated RCM lysozyme, no activation can be observed (not shown). On the other hand, *p*-nitrophenol and phosphate (up to a concentration of 2 mM) do not inhibit the activation by PTPA (not shown), nor do they inhibit the PTPA-activated phosphatase activity

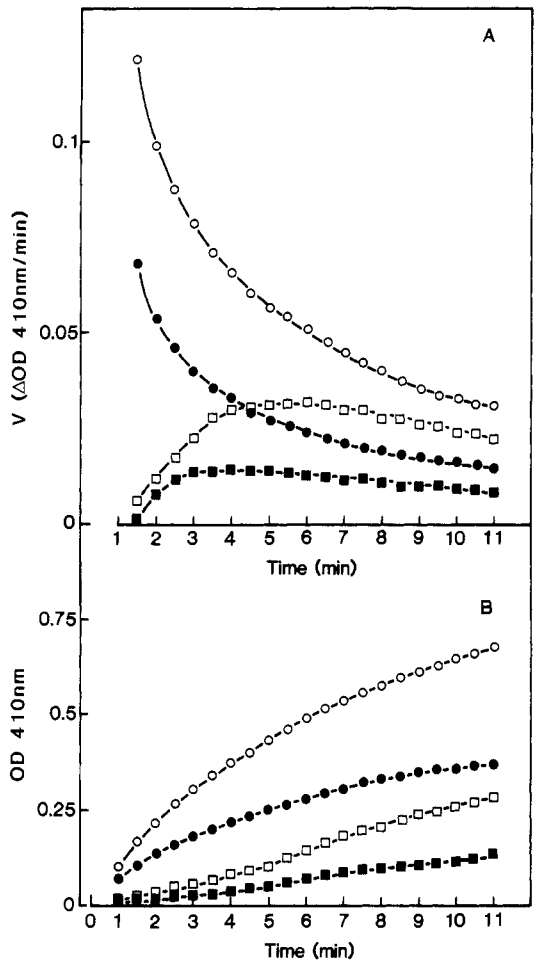

FIGURE 5: Kinetics of the *p*NPPase activity of the PCS_L phosphatase after activation by PTPA and stimulation by tubulin. Muscle PCS_L phosphatase was preincubated with (○, ●) or without (□, ■) saturating amounts of oocyte PTPA in the presence of ATP·Mg. After addition of the *p*NPP substrate mixture in the presence (○, □) or absence (●, ■) of 330 nM heat-treated tubulin, the OD at 410 nm was monitored every 30 s (panel B) and the ΔOD, calculated for each consecutive point of time, is shown in panel A.

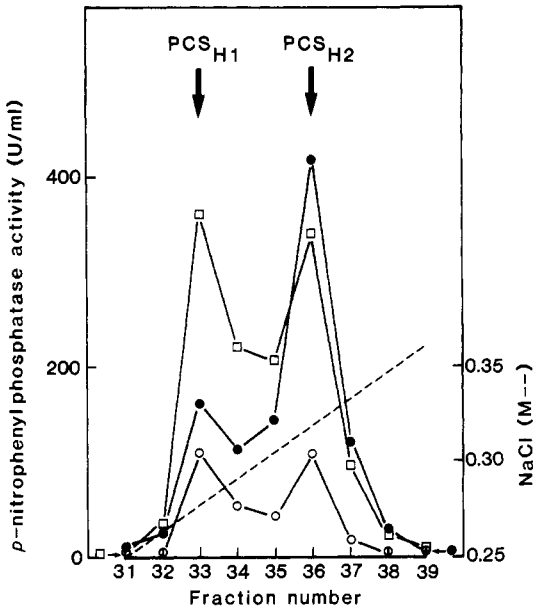

FIGURE 6: Specificity of the PTPA activation for the PCS_{H2} phosphatase. The figure shows the last purification step (Mono-Q HR 5/5 column) of the muscle PCS_H phosphatase as in Waelkens et al. (1987a). The fractions were assayed for the basal (○), ATP-stimulated (□), or PTPA-stimulated (●) *p*NPPase activity.

in concentrations up to 1 mM. These results clearly indicate that not the reaction products but the substrates inhibit the activation and presumably also induce deactivation.

(*C*) *Reversibility.* After complete activation of the aryl phosphatase activity of the PCS_L phosphatase by PTPA and removal of ATP·Mg by rapid gel filtration, a time-dependent decrease of the aryl phosphatase activity was observed ($t_{1/2}$ ±10 min); the activity could be restored by readdition of ATP·Mg. The aryl phosphatase could also be restored after the deactivation by *p*NPP. After the transient activation–deactivation and removal of the low molecular weight components by rapid gel filtration, a new activation cycle could be induced by ATP·Mg (results not illustrated).

(*D*) *Specificity for the PCS Phosphatases.* When the specificity of PTPA was tested with the different homogeneously purified PCS phosphatases from rabbit skeletal muscle and *Xenopus* oocytes, PTPA was remarkably specific toward the PCS_L and PCS_{H2} phosphatases, isolated from rabbit muscle or *Xenopus* oocytes.

The PCS_{H2} phosphatase is derived from the PCS_{H1} phosphatase by loss of its 55-kDa subunit (Waelkens et al., 1987a). The capability to be activated by PTPA is absent during the whole purification procedure of the PCS_H phosphatase (not shown). Only in the last purification step (Mono-Q), where the PCS_{H1} and PCS_{H2} phosphatases are separated, can the enzyme be activated in the fractions containing the PCS_{H2} phosphatase (Figure 6). Similar observations were made in the purification of the PCS_H enzyme from *Xenopus* oocytes.

By use of the PCS_C phosphatase purified to homogeneity (Ramachandran et al., 1987), PTPA and ATP·Mg could still bring about some PTPase activity, but the activity ratio seryl/tyrosyl phosphatase activity was 6-fold higher than for the PCS_L phosphatase. PTPA and ATP·Mg indeed activated the catalytic subunit and not some contaminating PCS_L phosphatase since in a Superose 12 column, the tyrosyl phosphatase activated by PTPA was located in the 35-kDa region, comigrating with the phosphorylase phosphatase activity (not shown).

The tyrosyl phosphatase activation of the PCS_L phosphatase does not appear to be an artifact of the purification procedure since both the activability as well as the PCS_L phosphatase are present from the first purification step onward (Figure 7). Once a crude *X. laevis* oocyte extract is separated on a DEAE-Sephacel column, different PTPase activities can be observed (Hendrix et al., 1989), but only these fractions, known to contain the PCS_L phosphatase, can be activated by PTPA and ATP·Mg (Figure 7). After activation, the PTPase activity observed represents at least 50% of the total cytosolic RCM lysozyme PTPase activity measurable at pH 7.5 in the presence of 20 mM Mg^{2+}. Roughly the same amount of PTPase activity can be found in the particulate fraction after Triton extraction, so that the total contribution to the total cellular PTPase activity can be estimated at 25%. The basal PTPase activity of the PCS phosphatases as measured by the dephosphorylation of RCM lysozyme at pH 7.5 is low, and the major PTPase activity elutes at a lower salt concentration (±180 mM NaCl). This activity (indicated as "1B" in Figure 7) shows many properties in common with the enzyme recently identified and isolated as PTPase 1B from human placenta (Tonks et al., 1988a) or with the enzyme previously described as PTPase T_2 in chicken brain (Foulkes et al., 1983). Characteristics tested so far include the following: pH optimum of 7.5 with RCM lysozyme, acid optimum of 5.5 with *p*NPP, the finding that the activity was blocked by 10 μM vanadate, the elution position during DEAE chromatography,

Table III: Comparison of the Characteristics of the Basal and ATP-, Tubulin-, or PTPA-Stimulated Activity of the PCS_L Phosphatase

	pH optimum[a]			$A_{50}(Mg^{2+})$ (mM)		polylysine I_{50} or A_{50} (μg/mL)	
	*p*NPP	RCM-Lys[e]	MLC	*p*NPP	RCM-Lys[e]	*p*NPP	RCM-Lys[e]
basal activity	9	9	9[b]	12	9	I_{50} 1.5–2	I_{50} 10–20
ATP stimulated	9	9	9[b]	12	9	I_{50} 1.5–2	I_{50} 10–20
tubulin stimulated	9	8	c	ND[d]	ND[d]	ND[d]	ND[d]
PTPA	8–9.5	7.5	7.5[b]	0.85	3	I_{50} 1.5–2	A_{50} 12

[a] In the presence of 20 mM $MgCl_2$. [b] In the presence of 8 μg/mL protamine. [c] No stimulation of tubulin was observed (see text). [d] Not determined. [e] RCM lysozyme.

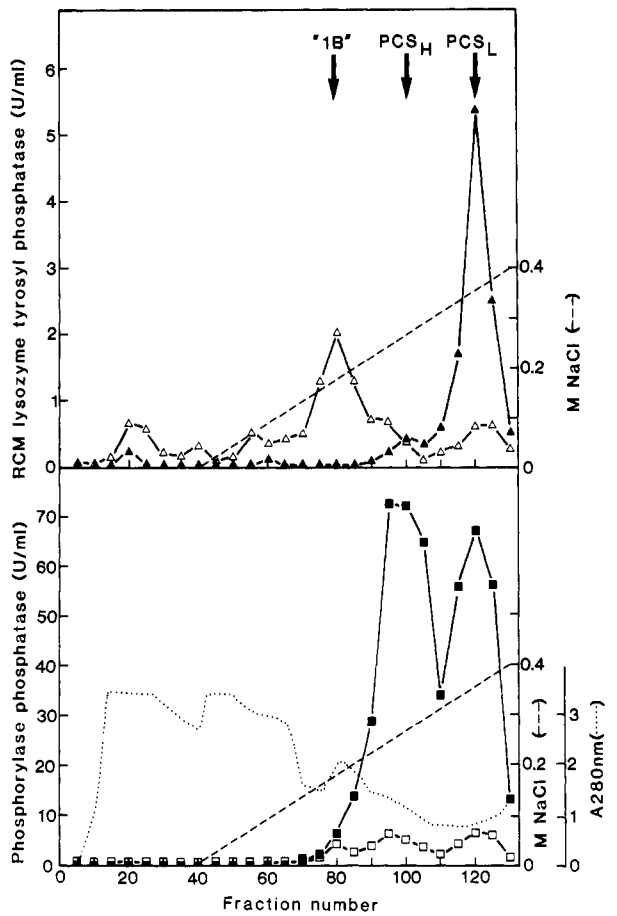

FIGURE 7: DEAE-Sephacel of a 100000*g* supernatant of *X. laevis* ovaries. A postribosomal supernatant of 63 g of *Xenopus* ovaries was prepared as in Hermann et al. (1988) and loaded on a DEAE-Sephacel column (2.5 × 15 cm) equilibrated in buffer A and the chromatography developed with a 2 × 500 mL (0–0.5 M NaCl) gradient in the same buffer. Fractions were assayed for phosphorylase phosphatase in the absence (□) and presence (■) of 33 μg/mL protamine and 15 mM $(NH_4)_2SO_4$ and for RCM lysozyme PTPase in the presence of 20 mM $MgCl_2$ with (▲) and without (△) PTPA activation.

and a low K_m value for RCM lysozyme (0.9 μM). The inhibition of this phosphatase by PTPA and ATP·Mg in the eluted fractions is seen also when the fractions were incubated with ATP·Mg alone (I_{50} = 0.15 mM ATP in the presence of 2.25 mM Mg^{2+}).

Only these fractions containing the PCS_L phosphatase were activated by PTPA and ATP·Mg (Figure 7) whether *p*NPP or a substrate phosphorylated on tyrosyl residues was used as substrate. None of the other alkaline or acidic *p*NPPases or PTPases detected in the DEAE-Sephacel eluate were stimulated by PTPA and ATP·Mg, nor the commercially available alkaline phosphatase from *E. coli* or calf intestine, nor the different forms of the AMD phosphatase (not illustrated).

(*A*) *Characteristics of the Activated Phosphatase.* (*A*) *Substrate Specificity.* Activation of the PCS_L phosphatase by PTPA and ATP·Mg leads to an increase of the aryl phosphatase activity, measured by its capacity to hydrolyze the phosphoester bound in *p*NPP, phosphotyrosyl RCM lysozyme, or phosphotyrosyl MLC. Since the PCS_L phosphatase was originally known as a Ser/Thr phosphatase (Waelkens et al., 1987a), we also tested the effect of PTPA (up to 100-fold the saturating concentration in the aryl phosphatase activation of the PCS_L enzyme) and ATP·Mg on the phosphorylase phosphatase activity of the PCS_L phosphatase. Neither the basal nor the polycation-stimulated phosphorylase phosphatase activity was affected. No substrate-induced inactivation could be observed in the progress curve of the phosphorylase phosphatase activity after a preincubation with PTPA and ATP·Mg (not shown). However, when nonradioactive phosphorylase *a* was added in the assay of PTPase with phospho-RCM lysozyme as the substrate, a strong inhibition by phosphorylase *a* (I_{50} = 5 μM), in contrast to that by phosphorylase *b* (I_{50} = 135 μM), was observed, as could be expected if both substrates compete for the same site. In none of the conditions used (basal, ATP stimulation, PTPA activation) could the PCS phosphatases dephosphorylate poly[Glu,Tyr (4:1)] at a pH range between 5 and 9.

(*B*) *Metal Ion and pH Dependency of the Activated Phosphatase* (*Table III*). The PTPase and *p*NPPase activities of the basal and ATP/PP_i-stimulated as well as PTPA-activated PCS_L phosphatase are completely dependent on the presence of Mg^{2+} or Mn^{2+} ions in the assay mixture. If EDTA is added to complex the Mg^{2+}, no phosphatase activity could be measured. However, the pH optimum for the PTPA-activated PTPase activity measured in the presence of Mg^{2+} is shift from pH 8.5–9 (for the basal and ATP/PP_i stimulated) to pH 7–7.5. With *p*NPP as the substrate, no real pH optimum could be determined for the PTPA-activated enzyme in the presence of 10 mM Mg^{2+}. A plateau was observed in the interval pH 8–9.5, and only 30% less activity was observed at pH 7 (not shown). As to the Mg^{2+} concentration dependency, the PTPA-activated enzyme behaved differently from the basal- or ATP/PP_i-stimulated activity, since the A_{50} for Mg^{2+} was estimated to be 3 mM for the PTPA-activated PTPase, when measured at its optimal pH of 7.5 or at pH 8.5, as compared with an A_{50} of 9 mM, found for the basal and ATP/PP_i-stimulated PTPase. When *p*NPP was used as the substrate, the change in the A_{50} for Mg^{2+} was even more drastic. After activation by PTPA, the A_{50} for Mg^{2+} was 0.85 mM and 12 mM for the basal and ATP/PP_i-stimulated activity [see Table III, Goris et al. (1988), and Hermann et al. (1988)]. The shift in pH optimum and A_{50} for Mg^{2+} would suggest the involvement of a different mechanism of catalysis by either the PTPA-activated or the ATP/PP_i-stimulated enzyme.

(*C*) *Other Effectors.* Polycations such as polylysine and protamine can stimulate the PTPase activity with RCM lysozyme or the MLC as substrates (Table III). However, the A_{50} is higher than with phosphorylase *a*, and the basal as well

as the ATP- and tubulin-stimulated PTPase activities are inhibited by these polycations. Also the PTPA-activated *p*NPPase is inhibited by polycations.

Orthovanadate, a known inhibitor of PTPases, inhibits the basal as well as the ATP- and PTPA-activated PTPase activity of the PCS_L enzyme with similar I_{50} values of about 0.6 mM when RCM lysozyme was the substrate.

Okadaic acid, a strong inhibitor of the phosphorylase phosphatase activity of the PCS phosphatases, with I_{50} values in the nanomolar concentration range and depending on the enzyme concentration (Bialojan & Takai, 1988; Haystead et al., 1989; Goris et al., 1989b), is also a strong inhibitor of the basal, ATP-stimulated-, or PTPA-activated aryl phosphatase activities of the PCS_L phosphatase whether phospho-RCM lysozyme, phospho-MLC, or *p*NPP is used as substrate. I_{50} values are in the same nanomolar concentration range as with phosphorylase *a* as the substrate and depend also on the enzyme concentration used in the assay (not illustrated).

(5) Comparison of the PTPA-Activated with the ATP/PP_i- and Tubulin-Stimulated PTPase Activity of the PCS_L Phosphatase. So far, three different types of effectors have been found to stimulate the PTPase activity of the PCS_L phosphatase. The effects of tubulin and ATP/PP_i are additive (Jessus et al., 1989), and therefore, both mechanisms of stimulation are probably different. When the PCS_L phosphatase is fully activated by PTPA, tubulin can bring about an extra stimulation of the *p*NPPase (Figure 5) or P-Tyr RCM lysozyme phosphatase activity (not shown). As can be seen in the progress curve of the *p*NPPase assay (Figure 5), the activity of the enzyme activated by both effectors levels off during the assay toward a stable reaction rate, the same as if the phosphatase were stimulated by tubulin alone. Thus tubulin cannot prevent the substrate-induced deactivation of the PTPA-activated enzyme, and as a result of the PTPA-induced activation the substrate does not cause a decrease in the tubulin-stimulated activity of the enzyme. Apparently, both activation processes are additive and independent. In addition to this, a different pH optimum for the tubulin-stimulated and PTPA-activated *p*NPPase or PTPase was observed (Table III). Using P-Tyr MLC as the substrate, no tubulin effect could be observed, but this might be due to the nature of this substrate. Indeed, PTPase activity with this substrate is highly dependent on the presence of polycations (not shown), and since there exists an interaction between tubulin and polycations such as protamines or polylysine (Jessus et al., 1989), the tubulin effect could be canceled by the (almost essential) polycations. The ATP/PP_i-stimulated PCS_L phosphatase could further be activated by PTPA in the presence of ATP·Mg. The reverse experiment, in which the effect of ATP would be followed after the activation by PTPA in the presence of ATP·Mg, was technically impossible, since Mg^{2+} cancels the ATP/PP_i activation (Hermann et al., 1988; Goris et al., 1988). The three mechanisms for inducing PTPase activity also appear to have a different specificity toward the respective PCS phosphatases. Whereas ATP and PP_i can stimulate all the different forms, tubulin stimulation is limited to the PCS_L phosphatase and PTPA activation is specific for the PCS_{H2}, PCS_L, and PCS_C phosphatase. As a general conclusion, we can say that the three mechanisms of activation discussed seem to be different, independent, and additive.

Discussion

Through the discovery of the ATP/PP_i-mediated PTPase activation of the PCS phosphatases (Hermann et al., 1988; Goris et al., 1988) we became aware of the possible regulation of the tyrosyl phosphatase activity of the PCS phosphatases. The first, more physiological effectors appeared to be the microtubular proteins tubulin and MAP_2 (Jessus et al., 1989). This type of regulation of activity and specificity of the phosphatase activity implies a new role for the spatial reorganization of microtubuli during mitosis or other cellular events, through the control of local phosphatase activity and its substrates. The regulation of the PTPase activity by PTPA could be even more important. After PTPA-induced activation of the PCS_L phosphatase, the PTPase activity is in the same order of magnitude as its phosphorylase phosphatase activity, the optimal pH is near neutrality, and the Mg^{2+} dependency (A_{50} = 3 mM) is below the 10 mM concentration of Mg^{2+} estimated in vivo (Sols & Marco, 1970). After activation by PTPA, the PTPase activity associated with the PCS_L phosphatase represents an important fraction (about 50%) of the measurable cytosolic PTPase activity with P-Tyr RCM lysozyme as the substrate. Routine measurements of the PTPase activity were carried out with 1 μM RCM lysozyme as the substrate. With a 10-fold higher concentration the activity was almost 10-fold higher and therefore was still below saturation. Hence the specific PTPase activity of the PCS_L phosphatase might be underestimated. Attempts to measure accurate K_m values for the PTPA-activated enzyme failed, probably because of the rapid deactivation of the PTPase activity during the assay, so that initial rates cannot be measured.

Since the activity changes induced by PTPA in the presence of ATP·Mg are only observed with aryl substrates and not with phosphorylase, the question can be asked whether both activities reside in the same active site, or on two active sites of the same polypeptide chain, or in two different enzymes, present in the same preparation. Moreover, the question can be asked whether the PTPA-activated PTPase activity resides in the PTPA molecule, brought out by an incubation with the PCS phosphatase and ATP·Mg, or in the PCS phosphatase. Our data support the hypothesis that a unique enzyme site shares both the PTPA-activated aryl phosphatase and the alkyl phosphatase activities: (1) both activities copurify with the PCS_L phosphatase up to apparent homogeneity; (2) the PTPase activity appearing in the last purification step of the PCS_H type phosphatase copurifies with the PCS_{H2} phosphatase and cannot be separated from this enzyme; (3) the possibility to be activated by PTPA is also associated—to a lesser extent—with the PCS_C phosphatase; (4) the PTPA-activated PTPase as well as the phosphorylase phosphatase activities are stimulated by polycations and inhibited by very similar concentrations of okadaic acid; (5) the PTPA-activated PTPase activity is inhibited by phosphorylase *a*; (6) the activity resulting from the incubation of different ratios of phosphatase and activator (Figure 3) is highly suggestive for the presence of the PTPase activity in the PCS phosphatase rather than in the PTPA; (7) the PTPase activity of the PCS phosphatase can also be brought out in the absence of PTPA by ATP or PP_i (Hermann et al., 1988; Goris et al., 1988) and tubulin (Jessus et al., 1989).

The mechanism of activation is not fully understood and needs further investigation. The requirement of ATP·Mg as cofactor for the activation is very suggestive for a kinase reaction. This hypothesis is still strengthened by the fact that nonhydrolyzable ATP analogues, as well as ADP and AMP, are ineffective. However, no phosphate incorporation could be found during the activation reaction, neither in the PCS phosphatase proteins nor in the PTPA protein. One could argue that a rapid (auto)dephosphorylation reaction could

possibly be the reason, since indeed P-Ser/Thr phosphatase as well as P-Tyr phosphatase can be ascribed to the activated enzyme. But neither short incubation times, with high concentrations of PTPA, nor the use of labeled ATPγS could reveal any incorporation in either the activator or the phosphatase. These observations are reminescent of the activation of the ATP·Mg-dependent phosphatase by kinase F_A, where originally (Yang et al., 1980) no correlation between phosphate incorporation and activation could be found. This could be explained subsequently by a transient phosphorylation of the modulator subunit, resulting in the activation of the catalytic subunit (Hemmings et al., 1982; Resink et al., 1983; Jurgensen et al., 1983, 1984; Ballou et al., 1983).

The shift in pH curves and the different concentration dependency of Mg^{2+} ions of the PTPA-activated PTPase would suggest a different mechanism of catalysis by the PTPA-activated enzyme. A possible explanation is that a conformational change of the PTPA-activated enzyme could increase the activity toward aryl phosphates at the more physiological pH while making the reaction less dependent on the still essential metal ions without affecting the activity toward the structurally different alkyl phosphates. It cannot be excluded that, through the PTPA activation, a completely new site is opened, which has no alkyl phosphatase activity but which might be in the proximity of the alkyl phosphatase site. During the catalysis the enzyme would revert to its original conformation, explaining the reversible deactivation observed after addition of the substrate. However, the ratio between the amount of enzyme in the assay (10 nM at 1 μg/mL or 0.3 pmol in 30 μL) and the amount of substrate converted excludes the possibility that the enzyme reaction itself brings about the reversible inactivation. Therefore, we rather suggest that the PTPA-activated [enzyme–substrate] complex is unstable. The time course of the *p*NPP phosphatase reaction by the nonactivated, tubulin-stimulated (Figure 5), or the ATP/PP_i-stimulated enzyme (not shown) also exhibits a pronounced lag. Such an observation may also be indicative of an induced-fit conformational change, enhancing the activity of the phosphatase. No lag period is observed when the RCM lysozyme or the MLC are used as phosphotyrosyl substrates.

The specificity of PTPA for the two-subunit forms (PCS_L and PCS_{H2}) and catalytic subunit of the PCS phosphatases is remarkable. It shows that the PTPase activity brought out by PTPA activation is localized on the 35-kDa catalytic subunit and that the 65-kDa subunit, common to the different PCS phosphatases, does not prevent but rather stabilizes or facilitates the activation reaction as indicated by the much higher PTPase/phosphorylase phosphatase activity ratio (after activation) observed with the PCS_L and PCS_{H2} phosphatases than with the PCS_C phosphatase. Apparently in the PCS_{H1} and PCS_M phosphatases other subunits (e.g., the 55-kDa subunit in the PCS_{H1} and the 72-kDa subunit in the PCS_M phosphatase) can prevent the activation reaction. This could be due to steric hindrance, occupancy of the binding sites for PTPA, or allosteric prevention of the conformational change of the catalytic subunit. This is clearly illustrated in the case of the PCS_{H1} and PCS_{H2} phosphatases, where the PTPA-induced PTPase activation becomes possible after removal of the 55-kDa subunit. A physiological function of the 55-kDa (in the PCS_{H1}) and 72-kDa (in the PCS_M phosphatase) subunit might therefore be to prevent the expression of the Tyr phosphatase activity.

It is not known whether there are in vivo situations in which the 55-kDa subunit of the PCS_H phosphatase could dissociate. If this were the case, this could lead to the expression of a substantial PTPase activity, maybe about the same amount of PTPase activity as in the cytosol of oocytes. Therefore, it seems not unreasonable to assume that in addition to its Ser/Thr phosphatase specifying role (Imaoka et al., 1983) the functional role of the 55-kDa subunit in the PCS_{H1} phosphatase (and the 72-kDa subunit in the PCS_M phosphatase) is to suppress the PTPase activity and that there is some type of regulation at the level of the noncatalytic subunits.

It has been suggested by Ingebritsen et al. (1983) and Tung et al. (1985) that the PCS_L ($=2A_2$) phosphatase may not exist in tissue extracts. This assumption was based on the combination of three arguments: (1) Partially purified preparations of PCS_H ($=2A_1$) phosphatase from rabbit liver undergo partial dissociation to PCS_L ($=2A_2$) phosphatase when rechromatographed on DEAE-cellulose. (2) PCS_L ($=2A_2$) phosphatase was not detected if the pH 6.1 supernatant from muscle was first adsorbed batchwise to DEAE-Sepharose. (3) Dissociation to phosphatase $2A_2$ does not occur during the further purification of phosphatases $2A_0$ or $2A_1$. Since we know that two kinds of two-subunit PCS phosphatases exist (PCS_{H2} and PCS_L) with different substrate specificity (Goris et al., 1986) and that PCS_{H2} can be generated from PCS_{H1} phosphatase by losing its 55-kDa subunit, it is more likely that the phosphatase dissociating from the phosphatase $2A_1$ by rechromatography on DEAE-cellulose was the PCS_{H2} phosphatase and not the $PCS_L/2A_2$ phosphatase. We always find the PCS_L phosphatase from the first purification step (DEAE) onward, and the PCS_{H2} phosphatase specifically originates from the PCS_H phosphatase in the last purification step (Mono-Q column) of muscle as well as oocyte PCS_H phosphatase. Therefore, Ingebritsen et al. (1983) probably lost the PCS_L phosphatase during the alternative purification procedure rather than avoiding the generation of the PCS_L phosphatase.

The observation that ATP·Mg can inactivate the major spontaneously active PTPase in the DEAE-Sephacel column eluate of oocyte extracts (Figure 7) might be important. On the basis of all the characteristics tested so far we can assume that this enzyme is the oocyte homologue of PTPase 1B from human placenta (Tonks et al., 1988a,b). This means that this phosphatase is inactivated in the presence of ATP·Mg, probably through the action of another enzyme and not by a direct effect on the phosphatase, since the inactivation by ATP·Mg is not observed with the purified placental PTPase 1B [see Table III in Tonks et al. (1988b)]. This observation is currently further investigated.

The apparent structural relationship between placental phosphatase 1B and the cytoplasmic domains of leukocyte common antigen CD45 (Charbonneau et al., 1988), and the demonstration that this antigen is a PTPase (Tonks et al., 1988c), opens new perspectives. Since CD45 antigen is a transmembrane protein located at the plasma membrane, with an extracellular domain that could function as a receptor for a ligand which has yet to be identified, it has been suggested that intracellular PTPase activity may be regulated by ligand binding. These results, together with our observations, strongly support the idea that protein tyrosine phosphatases are regulated enzymes. The relative importance of both phosphatase systems in the dephosphorylation of cellular tyrosyl phosphorylated substrates remains to be determined. For the time being, the proteins phosphorylated on tyrosyl residues in vivo are practically unknown, but on the basis of the limited information concerning in vitro substrates, the PCS phosphatases have a rather broad PTPase substrate specificity: the four sites of the EGF receptor, the *src* peptide, corresponding to one of the autophosphorylation sites of the *src* kinase (Goris et al.,

1988), P-tyrosyl MLC, and the artificial RCM lysozyme are all dephosphorylated by the PCS phosphatases, and for the *src* peptide and RCM lysozyme they represent a major activity in *Xenopus* oocytes. In contrast with the phosphatase "1B-like" activity in oocytes, the PCS phosphatases are not able to dephosphorylate poly[Glu,Tyr, (4:1)].

The observation that phosphorylase *a* is a much more potent inhibitor (I_{50} = 5 μM) of the PTPA-activated PTPase of the PCS_L phosphatase than phosphorylase *b* (I_{50} = 135 μM) is not only important as an argument for the involvement of the same active domain for both dephosphorylation (Tyr and Ser/Thr) reactions but could also have important physiological consequences. Indeed, the in vivo concentration of phosphorylase has been estimated at 80 μM in skeletal muscle (Cohen, 1978) and 11 μM in liver (Roesler & Khandelwal, 1986), and dephosphorylation of phosphorylase *a* to a certain level might therefore be a prerequisite before the PCS phosphatase can act as a PTPase, in analogy with the inhibitory action of phosphorylase on the synthase phosphatase activity in liver [see Stalmans et al. (1987)]. The implication might be that this represents a link between the major metabolic pathways and the mitotic signaling.

Since 10 μM okadaic acid did not inhibit PTPases in a crude 3T3 cell lysate using various phosphotyrosine-containing substrates such as enolase, poly(Glu,Tyr) and in vitro autophosphorylated cell lysates (Bialojan & Takai, 1988), and since 1 μM okadaic acid also did not inhibit the *Xenopus* homologue of the 1B PTPase (Hendrix et al., 1989), the specific inhibition of the PTPase activity by low concentrations of okadaic acid, under conditions that allow the expression of this activity by the PCS phosphatases, might be a helpful tool in discriminating between the PTPase activity of the PCS phosphatases and that of other enzymes. Injection of okadaic acid into *Xenopus* oocytes leads to germinal vesicle breakdown and the formation of active maturation-promoting factor within 60 min (Goris et al., 1989b). It is not known whether inhibition of a PTPase is indeed important as an explanation of these observations, but it would certainly fit with the hypothesis of the central role for tyrosyl phosphorylation in the mitotic signaling. It would also be in line with the recent observation (Draetta et al., 1988) that the CDC2 protein kinase, which is implicated in the maturation-promoting factor activity [see Dunphy and Newport (1988) for a recent review], is a major tyrosine kinase substrate and that its phosphotyrosine content is subject to cell cycle regulation.

The existence of PTPA in the cytosol of oocytes from amphibia, as well as in highly differentiated cells (muscle, liver) from mammalia, is highly suggestive for a conserved function for PTPA in the cells of the animal kingdom. Its cellular concentration is sufficiently high to play an important role in the regulation of the PCS_L phosphatase. After activation this phosphatase becomes a major PTPase with a pH optimum near neutrality and a specific activity in the same range as its phosphoseryl/threonyl phosphatase activity. Its special feature of being deactivated by the phosphotyrosyl substrate makes it an extremely interesting device for regulation: its effect can rapidly be quenched when phosphorylation on tyrosyl residues is required. A coordinated inactivation of PTPA itself would prohibit a futile cycle in this condition. Further investigation of the role of PTPA as a possible and ubiquitous cellular safety device to keep tyrosyl phosphorylation under strict control is clearly a primary challenge.

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Supplementary Material Available

A detailed description of the materials and methods used, including the assays, as well as the different purification procedures and additional references (8 pages). Ordering information is given on any current masthead page.

Registry No. PTPase, 79747-53-8; Mg-ATP, 1476-84-2.

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Effect of Nucleotide Substitution on the Peptidyltransferase Activity of 2′(3′)-*O*-(Aminoacyl) Oligonucleotides†

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ABSTRACT: Seven 2′(3′)-*O*-(aminoacyl) trinucleotides with structures derived from the 3′-terminal C-C-A sequence of aa-tRNA via nucleotide substitutions were investigated as acceptor substrates in the peptidyltransferase reaction and as inhibitors of substrate binding to the peptidyltransferase A site. It was found that all tested compounds were active in both systems, although substitution in the first and second nucleotide position results in some decrease of acceptor activity. Remarkably, replacement of natural cytidylic acid residues in C-C-A-Phe with guanylic acid moieties resulted only in a small decrease of acceptor or binding activity. The results indicate that the acceptor sequence of aa-tRNA is not probably engaged in base pairing with a sequence of 23S RNA during its interaction with the peptidyltransferase A site.

The common 3′-terminal C-C-A[1] sequence of aa-tRNA[1] and peptidyl-tRNA specifically interacts with acceptor and donor sites of peptidyltransferase during the peptide chain elongation process on the ribosome. Simple analogues of the 3′-terminus of aa- and peptidyl-tRNA, such as 2′(3′)-*O*-(aminoacyl) or peptidyl nucleosides and oligonucleotides, are capable of interacting with peptidyltransferase A and P sites and participate

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[1] Abbreviations: aa-tRNA, aminoacyl transfer ribonucleic acid; A-Gly, 2′(3′)-*O*-glycyladenosine (similar abbreviations are used for oligonucleotide derivatives); A_{260} unit, quantity of material contained in 1 mL of solution that has an absorbance of 1.00 at 260 nm when measured in a 1-cm path-length cell; poly(U), poly(uridylic acid); $tRNA^{Phe}_{yeast}$, transfer ribonucleic acid from yeast, specific for phenylalanine. Standard abbreviations for nucleosides and oligonucleotides are according to CBN recommendations (Sober, 1970).

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