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Biochemistry

An acid phosphatase in the plasma membranes of human astrocytoma showing marked specificity toward phosphotyrosine protein

(phosphotyrosine histone phosphatase/vanadate inhibition)

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ABSTRACT **The plasma membrane from the human tumor astrocytoma contains an active acid phosphatase activity based on hydrolysis of *p*-nitrophenyl phosphate. Other acid phosphatase substrates—β-glycerophosphate, *O*-phosphorylcholine, and 5′-AMP—are not hydrolyzed significantly. The phosphatase activity is tartrate insensitive and is stimulated by Triton X-100 and EDTA. Of the three known phosphoamino acids, only free *O*-phosphotyrosine is hydrolyzed by the membrane phosphatase activity. Other acid phosphatases tested from potato, wheat germ, milk, and bovine prostate did not show this degree of specificity. The plasma membrane activity also dephosphorylated phosphotyrosine histone at a much greater rate than did the other acid phosphatases. pH profiles for free *O*-phosphotyrosine and phosphotyrosine histone showed a shift toward physiological pH, indicating possible physiological significance. Phosphotyrosine histone dephosphorylation activity was nearly 10 times greater than that seen for phosphoserine histone dephosphorylation, and K_m values were much lower for phosphotyrosine histone dephosphorylation (0.5 μM vs. 10 μM). Fluoride and zinc significantly inhibited phosphoserine histone dephosphorylation. Vanadate, on the other hand, was a potent inhibitor of phosphotyrosine histone dephosphorylation (50% inhibition at 0.5 μM) but not of phosphoserine histone. ATP stimulated phosphotyrosine histone dephosphorylation (160–250%) but inhibited phosphoserine histone dephosphorylation (95%). These results suggest the existence of a highly specific phosphotyrosine protein phosphatase activity associated with the plasma membrane of human astrocytoma.**

Since the discovery of cAMP-dependent protein kinase (1) in 1968, the importance of reversible phosphorylation as a means of enzyme regulation has been of paramount interest to biochemists. The role of membrane phosphorylation in viral transformation and the mechanism of action of epidermal growth factor have come to light only recently (2–7). Of primary importance in this event has been the discovery of protein kinase activities that will specifically catalyze the phosphorylation of other proteins at tyrosine residues (2–7).

The importance of tyrosine phosphorylation in growth and neoplastic transformation and the possible regulation of these events by dephosphorylation has initiated a search for specific phosphotyrosine protein phosphatase activity. Carpenter *et al.* (8) were the first to suggest the existence of a putative dephosphorylating activity when they observed that phosphorylation by epidermal growth factor receptor in A431 epidermoid carcinoma cells quickly decreases after an initial burst of phosphorylation. Brautigan *et al.* (9) and Gallis *et al.* (10) indicated that Zn^{2+} inhibition at micromolar concentration is an intrinsic property of specific phosphotyrosine protein phosphatases. Swarup *et al.* (11) reported the selective dephosphorylation of phosphotyrosine protein substrates by alkaline phosphatases.

We recently identified a high acid phosphatase activity associated with the plasma membrane of the transplantable human tumor astrocytoma (12, 13). Various reports have appeared in the literature about membrane-associated acid phosphatase activity, but they were usually associated with the lysosomal membrane (14–16). The acid phosphatase activity in the astrocytoma plasma membrane cosediments in sucrose gradients with well-established plasma membrane marker enzymes (5′-nucleotidase and Mg^{2+}-ATPase) and with sialic acid.

Because of the similarity in structure between *p*-nitrophenyl phosphate and phosphotyrosine, we undertook a study to determine if the astrocytoma plasma membrane phosphatase activity would preferentially dephosphorylate protein substrates containing phosphotyrosine. We report here that the astrocytoma plasma membrane acid phosphatase activity is selective for protein-bound phosphotyrosine.

MATERIALS AND METHODS

Materials. *O*-Phosphotyrosine, phosphoserine, phosphothreonine, *p*-nitrophenyl phosphate, Triton X-100, histone type IIA, and acid phosphatases from wheat germ (type I), potato (type IV), milk (type V), and bovine prostate (type VI) were obtained from Sigma. [γ-^{32}P]ATP (3,000 Ci/mmol; 1 Ci = 3.7 × 10^{10} becquerels) was obtained from Amersham. A431 human epidermoid carcinoma cells were kindly provided by Tomoyuki Kawamoto (University of California at San Diego). The catalytic subunit of cAMP-dependent protein kinase was a gift from Susan Taylor and Norman Nelson (University of California at San Diego). Sodium vanadate was obtained from Fisher.

Preparation of Subcellular Fractions. The three transplantable human tumors, a grade IV astrocytoma (T24), and two melanomas (T355 and T354) were maintained by serial transplantation in BALB/*nu* athymic mice as described (17). Plasma membrane was isolated and purified as described by Knowles *et al.* (12). Mouse liver lysosomes were isolated by the method of Trouet (18). A431 epidermoid carcinoma plasma membranes rich in epidermal growth factor receptor were purified by the method of Thom *et al.* (19).

Preparation of Phosphorylated Histones. Phosphorylation of histones at tyrosine residues was carried out by a modification of the procedure of Swarup *et al.* (11) in that the phosphorylation reaction was incubated in the presence of 100 μM sodium vanadate at 37°C for 20 min. Phosphorylation on serine residues in histones was carried out by using the catalytic subunit of the cAMP-dependent protein kinase under the following conditions. Ten milligrams of histone (type IIA) was incubated in 100 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.5/1 mM dithiothreitol/25 mM $MgCl_2$ containing 100 units of protein ki-

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nase and 200 μM [γ-^{32}P]ATP (1 × 10^8 cpm) for 1 hr at 37°C. The reaction was terminated by addition of trichloroacetic acid and the phosphorylated histones were recovered as described by Swarup *et al.* (11). Phosphorylated amino acid residues on the histones were identified after hydrolysis with 6 M HCl under reduced pressure at 105°C for 3.5 hr followed by thin-layer chromatography as described (11).

Enzyme Assays. All enzyme assays were carried out at 37°C. *p*-Nitrophenyl phosphatase activity was assayed as described (12). Hydrolysis of β-glycerophosphate, *O*-phosphorylcholine, and 5'-AMP was assayed in a 1-ml reaction mixture containing 0.1 M sodium acetate (pH 5.0), the indicated substrate at 10 mM, and 0.2% Triton X-100. The reaction was stopped by addition of trichloroacetic acid, and aliquots of the centrifuged supernatant were taken for P_i determination by the procedure of Lohmann and Jendrassik (20).

In the standard assay, hydrolysis of phosphotyrosine, phosphoserine, and phosphothreonine was determined in 0.5 ml of reaction mixture containing 0.1 M sodium acetate (pH 6.0), 10 mM substrate, 0.2% Triton X-100, and 1 mM EDTA. P_i released was determined as above.

Phosphoprotein phosphatase activity with phosphotyrosine histone and phosphoserine histone as substrates was assayed at 37°C in 0.1 M sodium acetate, pH 6.0/0.2% Triton X-100/1 mM EDTA in a total volume of 50 μl with the indicated amount of substrate and for the indicated length of time. The reaction was stopped by addition of 150 μl of 25% (wt/vol) trichloroacetic acid and immediately placed at 4°C for 15–30 min. Then, 50 μl of bovine serum albumin (10 mg/ml) was added as a carrier. After centrifugation (12,000 × *g* for 15 min) 100–150 μl of the supernatant was assayed for radioactive $^{32}P_i$ in a liquid scintillation counter. In some experiments $^{32}P_i$ was extracted from the supernatant by the procedure of Pullman (21) in order to decrease the background and the trichloroacetic acid quenching.

The amount of $^{32}P_i$ released from the histones and the [^{32}P]histone concentration were determined from the specific activity of the [γ-^{32}P]ATP used in the histone phosphorylation reaction.

RESULTS

Acid Phosphatase Activity of the Astrocytoma Plasma Membrane. Purified plasma membranes from two human melanoma lines (T355 and T354) and human astrocytoma (T24) were assayed for acid phosphatase activity on various reported acid phosphatase substrates. Mouse liver lysosomes were also assayed side by side for comparison. The astrocytoma plasma membrane acid phosphatase activity was selective for *p*-nitrophenyl phosphate as substrate; β-glycerophosphate was hydrolyzed at a much reduced rate (Table 1). The two melanoma lines tested also had high acid phosphatase activity associated with their plasma membranes; however, the activities toward both β-glycerophosphate and 5'-AMP were also high. Mouse liver lysosomal acid phosphatase showed a substrate hydrolysis profile similar to that seen for the melanoma plasma membranes but differed considerably from that shown by the astrocytoma plasma membrane.

The astrocytoma plasma membrane acid phosphatase activity also differed from the other three activities with regard to the effect of inhibitors on the hydrolysis of *p*-nitrophenyl phosphate. Tartrate at 10 mM had little effect on hydrolysis by the astrocytoma plasma membrane but inhibited the other three activities considerably (Table 2). Fluoride at 10 mM inhibited the astrocytoma plasma membrane activity only 70% but completely inactivated the other three activities. Other inhibitors of acid phosphatase or *p*-nitrophenyl phosphatase showed little effect (14). The acid phosphatase activity measured by the hydrolysis of *p*-nitrophenyl phosphate showed a sharp pH optimum at pH 5.5 (Fig. 1*a*). There was little phosphatase activity found in the pH range usually associated with alkaline phosphatase activity.

Table 1. Substrate specificity of acid phosphatase activity of tumor plasma membranes and mouse liver lysosomes

	Relative activity†			
	Astrocytoma	Melanoma		Mouse liver
Substrate*	T24	T355	T354	lysosomes
p-Nitrophenyl *P*	100 (0.984)	100 (0.553)	100 (1.234)	100 (0.487)
β-Glycero *P*	19	90	103	95
O-Phosphorylcholine	0	21	24	1
5'-AMP	8	65	79	34

Assays were carried out at 37°C in 1 ml of reaction mixture containing 0.1 M sodium acetate (pH 5.0), the indicated substrate at 10 mM, and 0.2% Triton X-100. *p*-Nitrophenyl phosphate hydrolysis was stopped by addition of 2 ml of 0.1 M NaOH, and absorbance at 410 nm was determined. The other reactions were stopped by addition of 0.1 ml of 50% (wt/vol) trichloroacetic acid. Aliquots of the centrifuged supernatant were taken for P_i determination by the method of Lohmann and Jendrassik (20).

* Substrate concentration in all cases is 10 mM.

† Value in parentheses is specific activity in μmol/min per mg of protein.

Hydrolysis of Free Phosphoamino Acids and Phosphotyrosine Histone Dephosphorylation. Table 3 shows the activity of the astrocytoma plasma membrane toward three phosphoamino acid substrates—phosphotyrosine, phosphoserine, and phosphothreonine—compared with the activity demonstrated by commercially available acid phosphatases from wheat germ, potato, milk, and bovine prostate. The activity from astrocytoma plasma membrane hydrolyzed phosphotyrosine but not phosphoserine or phosphothreonine. The acid phosphatase activities from wheat germ, potato, and bovine prostate did not demonstrate this type of selectivity. Although the activity from milk did not hydrolyze phosphoserine or phosphothreonine, it only dephosphorylated phosphotyrosine at a rate 32% of that seen for *p*-nitrophenyl phosphate and hydrolyzed phosphotyrosine histone only slightly.

Table 2. Effect of inhibitors and Triton X-100 on acid phosphatase activity of tumor plasma membranes and mouse liver lysosomes

	Relative enzyme activity*			
	Astrocytoma	Melanoma		Mouse liver
Inhibitor	T24	T355	T354	lysosomes
Triton X-100, 0.2%	100 (0.984)	100 (0.518)	100 (1.189)	100 (0.479)
Tartrate, 10 mM	97	43	21	14
Fluoride, 10 mM	30	4	1	3
Pyridoxine·HCl, 50 mM	104	73	57	92
PCMPS, 0.1 mM	69	78	59	62
Ouabain, 1 mM	98	97	97	104
Oligomycin, 10 μg	93	97	99	103

Activity was assayed at 37°C in 1-ml reaction mixture containing 0.1 M sodium acetate (pH 5.0) with 10 mM *p*-nitrophenyl phosphate as substrate. Effect of inhibitors on the activity was determined in the presence of 0.2% Triton X-100. PCMPS, *p*-chloromercuriphenylsulfonic acid.

* Value in parentheses is specific activity in μmol/min per mg of protein.

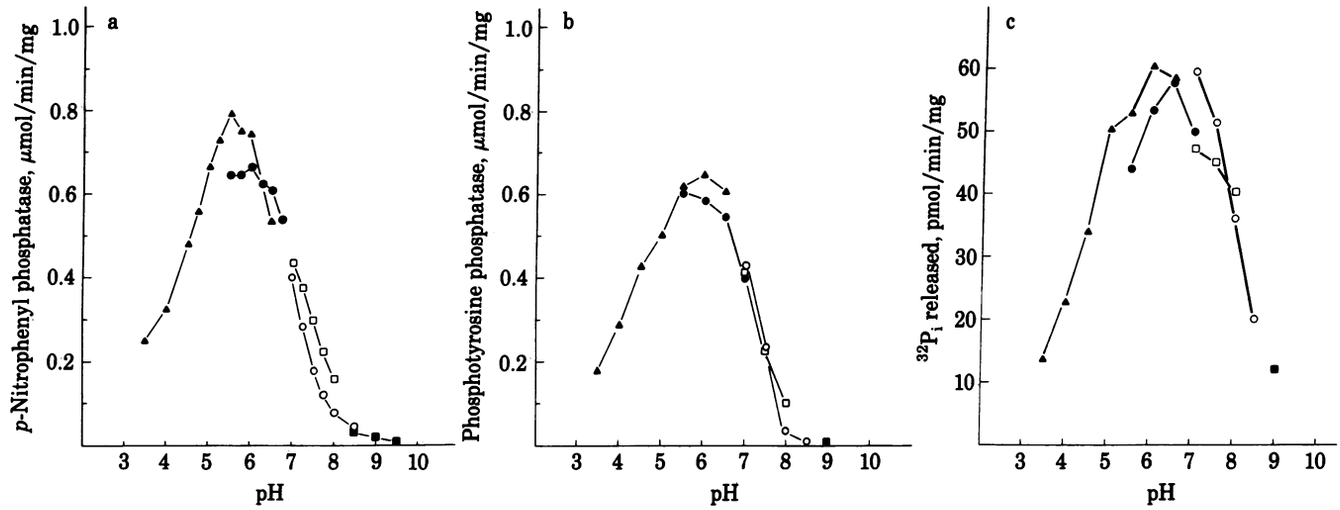

FIG. 1. pH profiles of hydrolysis of *p*-nitrophenyl phosphate (*a*) and phosphotyrosine (*b*) and dephosphorylation of phosphotyrosine histone (*c*) by astrocytoma plasma membrane. (*a*) Activities were determined at 37°C with 10 mM *p*-nitrophenyl phosphate as substrate, 0.2% Triton X-100, and the different buffers at different pH values as indicated. (*b*) Activities were determined at 37°C in 0.5 ml with 10 mM *O*-phosphotyrosine as substrate, 0.2% Triton X-100, 1 mM EDTA, and the indicated buffers. (*c*) Activities were determined in a volume of 50 μl with phosphotyrosine histone substrate (0.2 μM in radioactive phosphate), 0.2% Triton X-100, 1 mM EDTA, and the indicated buffers. Buffers: ▲, 50 mM sodium acetate, pH 3.5–6.5; ●, 50 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 5.5–6.75; ○, 50 mM Tris, pH 7.0–8.5; □, 50 mM Hepes, pH 7.0–8.0; ■, 50 mM glycine NaOH, pH 8.5–9.5.

Table 3 also shows the activity of the various phosphatases toward phosphotyrosine histone substrate. The astrocytoma plasma membrane dephosphorylated phosphotyrosine histone significantly better than the other acid phosphatases.

pH Optima. Fig. 1 *b* and *c* shows the pH profiles of the astrocytoma plasma membrane phosphatase activity toward free phosphotyrosine and phosphotyrosine histone, respectively. Hydrolysis of phosphotyrosine showed a pH optimum of pH 6.5, whereas the hydrolysis of phosphotyrosine histone showed a broad activity peak, ranging from pH 6.0 to pH 7.0. This shift in pH optimum from pH 5.5 for hydrolysis of *p*-nitrophenyl phosphate toward physiological pH for hydrolysis of phosphotyrosine histone may be indicative of a physiologically significant role for the astrocytoma plasma membrane acid phosphatase activity.

Table 3. Acid phosphatase activities from astrocytoma plasma membrane, wheat germ, potato, milk, and bovine prostate toward *p*-nitrophenyl phosphate, phosphorylated amino acid substrates, and phosphotyrosine histone

Acid phosphatase from	μmol/min/mg				nmol $^{32}P_i$ released/min/mg
	pNPP	Phosphotyrosine	Phosphoserine	Phosphothreonine	Phosphotyrosine histone
Astrocytoma plasma membrane	0.750	0.406	0.000	0.000	1.056
Wheat germ	0.324	0.333	0.109	0.110	0.088
Potato	1.520	1.445	0.421	0.450	0.354
Milk	0.338	0.109	0.000	0.000	0.002
Bovine prostate	0.004	0.004	0.003	0.002	0.006

Substrate concentration was 10 mM in assays with *p*-nitrophenyl phosphate (pNPP), phosphotyrosine, phosphoserine, and phosphothreonine and 1 μM with [^{32}P]phosphotyrosine histone. All enzyme assays were carried out at 37°C with a reaction mixture containing 0.1 M sodium acetate (pH 6.0), 0.2% Triton X-100, 1 mM EDTA, and the indicated phosphatase and substrate. Hydrolysis of pNPP was carried out as in Table 1. Assay for hydrolysis of phosphate from the phosphoamino acids was run in a volume of 0.5 ml, contained 10 mM substrate, and was stopped with trichloroacetic acid. P_i was determined as described (20). Phosphotyrosine histone phosphatase activity was assayed in a volume of 50 μl with the phosphotyrosine histone substrate 1 μM in ^{32}P. Incubation time varied for the different phosphatase activities so that <5% of substrate ^{32}P was released. In a typical experiment, incubation times were 30 sec, 1 min, 2 min, 10 min, and 5 min for astrocytoma plasma membrane, potato, wheat germ, milk, and bovine prostate acid phosphatase activities, respectively.

Phosphotyrosine Histone and Phosphoserine Histone Phosphatase Activities of the Astrocytoma Plasma Membrane. The astrocytoma plasma membrane phosphatase activity showed a marked preference for phosphotyrosine histone as substrate over phosphoserine histone. The membrane activity dephosphorylated phosphotyrosine histone at a rate nearly 9 times that for phosphoserine histone (Table 4).

The K_m values for phosphotyrosine histone and phosphoserine histone also suggest specificity. The K_m for phosphotyrosine histone dephosphorylation is nearly 1/20th that for phosphoserine histone dephosphorylation. It has been pointed out by Swarup *et al.* (11) that the A431 membranes contain very active *p*-nitrophenyl phosphatase and phosphoprotein phos-

Table 4. Activity of the astrocytoma plasma membrane enzyme on different substrates

Substrate	Specific activity		K_m, μM
	nmol $^{32}P_i$ released/min/mg	μmol/min/mg	
Phosphotyrosine histone	1.193	—	0.52
Phosphoserine histone	0.142	—	9.88
p-Nitrophenyl phosphate	—	0.780	640
O-Phosphotyrosine	—	0.434	2,940

Phosphotyrosine histone and phosphoserine histone phosphatase activities were assayed for 30 sec and 1 min, respectively, in 1 μM substrate. *p*-Nitrophenyl phosphate and *O*-phosphotyrosine activities were assayed as described in *Materials and Methods*. K_m values are the mean of at least two determinations and double-reciprocal plot analysis.

Table 5. Effect of phosphatase inhibitors on phosphotyrosine histone and phosphoserine histone phosphatase activities of astrocytoma plasma membrane

Inhibitor	Relative activity,* %	
	Phosphotyrosine histone	Phosphoserine histone
Triton X-100, 0.2%	100	100
EDTA, 5 mM	133	102
p-Nitrophenyl phosphate, 10 mM	82	80
Phosphotyrosine, 10 mM	66	38
Inorganic phosphate, 10 mM	73	44
Sodium fluoride, 10 mM	74	9
Zinc chloride, 100 μM	78	27
Sodium vanadate, 100 μM	11	96
ATP, 2 mM	159	5

The reaction was carried out for 1 min at 37°C after a 5-min preincubation with the indicated inhibitor at 23°C. Reaction mixture consisted of 100 mM sodium acetate (pH 6.0), 0.2% Triton X-100, phosphotyrosine and phosphoserine histone substrates at 1 μM in ^{32}P, and the indicated concentration of inhibitor.
* Based on $^{32}P_i$ released.

phatase activities. However, when the phosphorylation reaction was done in the presence of vanadate we were able to increase the amount of tyrosine histone phosphorylation so that phosphatase assays could be determined at substrate concentrations approaching 3 times the determined K_m. The K_m determinations were done by using concentrations from 100 nM to 1.4 μM in phosphotyrosine histone and 100 nM to 30 μM in phosphoserine histone. Linear regression analysis of double-reciprocal plots showed coefficients of correlation >0.99. K_m values were also determined for *p*-nitrophenyl phosphate and free *O*-phosphotyrosine and were >1,000-fold higher. These results indicate that the affinity for protein bound phosphate is much higher than that for small phosphate compounds.

Effect of Inhibitors on Astrocytoma Plasma Membrane Phosphatase Activity. Results in Table 5 demonstrate that significant differences between the effects of phosphatase inhibitors on phosphotyrosine histone and phosphoserine histone dephosphorylations. Fluoride, zinc, vanadate, and ATP all showed significant differential effects on the initial rate of histone dephosphorylation. Fluoride (10 mM) inhibited phosphotyrosine histone dephosphorylation 25%, whereas it almost totally inhibited phosphoserine histone dephosphorylation. Zn^{2+} inhibited phosphoserine histone dephosphorylation preferentially in our hands in contrast to published reports (9, 10). Vanadate (22) proved to be the most potent inhibitor of phosphotyrosine histone dephosphorylation (>90%) but inhibited phosphoserine histone dephosphorylation only slightly (4%). We encountered an interesting ATP effect on histone dephosphorylation. At 2 mM, ATP stimulated phosphotyrosine histone dephosphorylation (160–250%) but inhibited phosphoserine histone dephosphorylation almost completely. This result suggests the possibility that membrane phosphorylation may play a major role in the regulation of both dephosphorylating activities. The differential effects of inhibitors on the two activities also suggest the possibility that phosphotyrosine histone phosphatase and phosphoserine histone phosphatase activities may be due to different enzymes.

The effect of vanadate on the membrane phosphotyrosine histone phosphatase activity was studied further (Fig. 2). We found it to be a potent inhibitor of the astrocytoma plasma membrane phosphotyrosine histone phosphatase with 50% inhibition occurring at 0.5 μM vanadate. Dephosphorylation of *p*-nitrophenyl phosphate and free *O*-phosphotyrosine also was inhibited similarly at equal concentrations of vanadate.

DISCUSSION

These results suggest the existence of a highly specific phosphotyrosine protein phosphatase activity associated with the plasma membrane of human astrocytoma. It is significant that this activity is localized in the plasma membrane because protein kinases that phosphorylate at tyrosine have been shown to be localized in the plasma membrane (2, 4–7). The close proximity of phosphoprotein phosphatase activity allows for a means of regulating membrane phosphorylation.

Recent reports from several laboratories (9–11, 22) have demonstrated the possible existence of specific phosphotyrosine protein phosphatases. Swarup *et al.* (11) have shown that alkaline phosphatases from calf intestine, beef liver, and *Escherichia coli* show selective dephosphorylation of phosphotyrosine protein. Brautigan *et al.* (9, 10) demonstrated that phosphotyrosine protein phosphatase activity in epidermoid carcinoma A431 cells and rat embryo fibroblast N2 cells is specifically inhibited by Zn^{2+}. Our phosphotyrosine protein phosphatase activity differs significantly from these in several respects. The astrocytoma plasma membrane activity shows an acidic pH optimum based on hydrolysis of *p*-nitrophenyl phosphate unlike the alkaline phosphatase activities. Furthermore, our phosphotyrosine protein phosphatase activity is not inhibited in the presence of EDTA, *p*-nitrophenyl phosphate, or inorganic phosphate as was reported for alkaline phosphatase. We also found that, unlike the activities from A431 and N2 cells, the astrocytoma activity was only slightly inhibited by Zn^{2+} even at 10 times the concentration used in the above studies (9). In fact, we have been able to show that Zn^{2+} in micromolar concentrations will inhibit endogenous protein kinase activity in the astrocytoma plasma membrane.

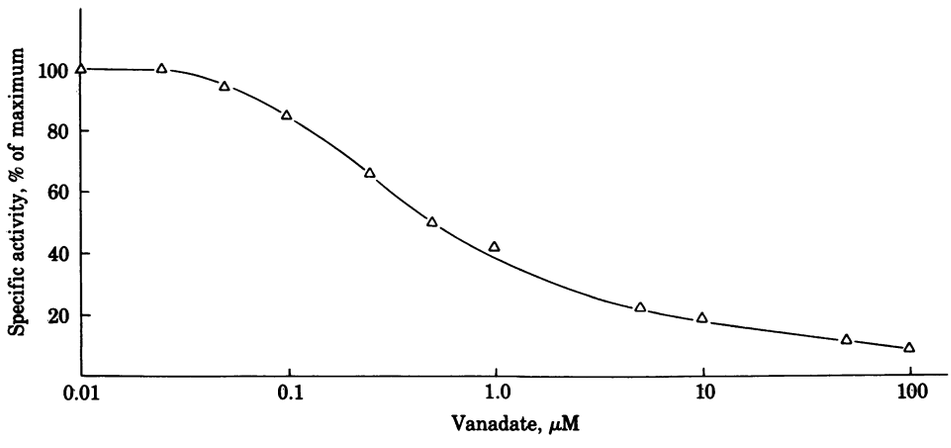

FIG. 2. Inhibition of phosphotyrosine histone phosphatase activity of the astrocytoma plasma membrane by vanadate. The reaction was run for 2 min at 37°C. The 50-μl reaction mixture consisted of 100 mM sodium acetate (pH 6.0), 0.2% Triton X-100, and radioactive phosphate phosphotyrosine histone substrate at 200 nM in ^{32}P, and the indicated concentration of sodium vanadate.

One of the more interesting results is the potent inhibitory effect of orthovanadate on phosphotyrosine protein phosphatase activity. Vanadate has been shown to be a potent inhibitor of Na^+,K^+-ATPase and other plasma membrane ATPases (23–25). A recent report by Smith and Brock (26) showed that vanadium is comitogenic with serum or insulin in quiescent Swiss 3T3 and 3T6 cells in the concentration range 5–50 μM. Vanadate by itself in serum-free medium increased [^{3}H]thymidine incorporation 3- to 5-fold. Several groups have shown that increased protein kinase phosphorylation at tyrosine by epidermal growth factor receptor, pp60^{src}, and other retrovirus onc gene products are essential for mitogenesis and transformation (2–7, 27). Micromolar concentrations of vanadate inhibit phosphotyrosine protein phosphatase activity and, in effect, could also increase the cellular phosphotyrosine concentration. This observation could explain the mitogenic effect of vanadate on the Swiss 3T3 and 3T6 cell types.

In order to justify phosphotyrosine-specific protein phosphatase activity in the plasma membrane of human astrocytoma one would expect to find a tyrosine phosphorylating protein kinase activity in the same membrane. Several reports have suggested that all cells have tyrosine phosphorylating activity in their plasma membranes (2, 4, 5). We have detected epidermal growth factor-insensitive, Mn^{2+}-dependent protein kinase activity in the astrocytoma membrane that is stimulated by glial growth factor (28).

The differential effects of inhibitors on astrocytoma plasma membrane phosphotyrosine protein phosphatase and phosphoserine protein phosphatase activities strongly suggest that the two activities are due to different enzymes. The difference in response of the two activities to ATP is also dramatic. One would not think it likely that the inhibitors would be acting differentially on the two phosphohistone substrates. Partial purification of the astrocytoma plasma membrane phosphatase activity by gel filtration on Sepharose CL-6B and ion exchange chromatography on DEAE-Sephadex has shown that *p*-nitrophenyl phosphate, *O*-phosphotyrosine, and phosphotyrosine histone dephosphorylating activities are eluted in the same fractions. Reports in the literature have shown that *p*-nitrophenyl phosphatase activity (29) can be copurified with phosphoprotein phosphatase activity and it remains to be seen if dephosphorylation of *p*-nitrophenyl phosphate is an intrinsic property of the astrocytoma plasma membrane phosphotyrosine protein phosphatase.

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