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Vanadium Compounds

Their Action on Alkaline Phosphatase Activity

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ABSTRACT

The direct effect of different vanadium compounds upon alkaline phosphatase (ALP) activity was investigated. Vanadate and vanadyl inhibited both the soluble and particulate ALP activity from UMR.106 cells and from bovine intestinal ALP. We have also shown the inhibition of ALP activity in the soluble fraction of osteoblasts by peroxo and hydroperoxo vanadium compounds. ALP activity in the particulate fraction was not inhibited by these species; nor was the bovine intestinal ALP. Using inhibitors of Tyr-phosphatase (PTPases), the soluble ALP was partially characterized as a PTPase. The major activity in the particulate fraction represents the bone-specific ALP-activity. This study demonstrates that different forms of vanadium are direct inhibitors of ALP activity. This effect is dependent on the enzymatic activity investigated and on the origin of the ALP.

Index Entries: Vanadium; alkaline phosphatase; tyrosine-phosphatase; osteoblast cells; bone cells; inhibitory effects.

INTRODUCTION

Vanadium is a transition metal of group 5 that shows various oxidation states. It is present in living systems in trace amounts (*1*). Vanadium affects different biological processes such as the inhibition of Na^+,

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K^+-ATPase, glucose oxidation in adypocites, insulin-mimetic actions, and the inhibition of several Tyrosine-phosphatases (PTPases) (*2,3*).

Phosphotyrosine accumulation is induced by vanadate inside the cultured cells (*4*). Vanadium +5 may be reduced to vanadyl +4 in the presence of NADPH and O_2 during cellular metabolism. Addition of peroxo- or hydroperoxide vanadium compounds to permeabilized HL-60 granulocytes greatly enhances the phosphotyrosine content of the cells (*4,5*). At present, the mechanism by which phosphotyrosine increases is unknown.

Osteoblast cells are involved in bone formation and their differentiation is modulated by multiple factors such as hormones, growth factors, and ions (*6*). Various peptide factors stimulate the tyrosine-kinases associated to the cognate receptors and subsequently they induce tyrosine-phosphorylation of specific cellular proteins. These effects are counterbalanced by the presence of the PTPases, which in turn deactivate the receptors (*7*). PTPases have been isolated and characterized in several systems. They belong to two general families: receptor-like PTPases associated with membranes, and nonreceptor-like PTPases (*8,9*).

The aim of this study was to investigate the direct effect of different vanadium compounds upon alkaline phosphatase (ALP) activity. We also attempted to characterize the enzymes sensitivity to vanadium from rat osteoblast cell line UMR.106.

METHODS

Materials

Vanadium (IV) oxide sulfate (vanadyl sulfate) was obtained from Merck, sodium o-vanadate, potassium superoxide, superoxide dismutase, p-nitrophenylphosphate (pNPP), and catalase were obtained from Sigma (St. Louis, MO), as well as the bovine intestinal alkaline phosphatase. Tissue culture materials were provided by Corning or Falcon. DMEM and trypsin-EDTA were supplied by Gibco (Gaithersburg, MD) and FBS by Gen (Argentina). All other chemicals used were of analytical grade from Sigma.

Solutions

Fresh stock solutions of vanadyl sulfate and sodium o-vanadate were prepared in distilled water at 100 mM concentration. Peroxovanadium and hydroperoxide vanadium compounds were prepared as previously reported (*4*).

Cell Culture

Rat osteosarcoma cell line (UMR.106) were grown in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine

serum (FBS) and antibiotics. Cells were passaged by Trypsin-EDTA treatment to 150 cm^2 flask once a week. After the cells reached confluence, the medium was removed and the monolayer washed with phosphate buffered saline (PBS) pH 7.4 and once in 50 mM Hepes/150 mM NaCl buffer containing 1 mM EDTA, 15 mM β-mercaptoethanol and 1 mM phenylethanosulfonyl fluoride *(10)*.

Preparation of Cellular Fractions

Cells were scraped in the above buffer and sonicated at 600 psi/15 s. The homogenate was centrifuged at 2500 rpm/5 min. The supernatant was centrifuged at 45,000 g/90 min at 4°C. The supernatant (soluble fraction) was stored at –70°C. The pellet was solubilized in 50 mM Hepes/150 mM NaCl/1% Triton-X100 and recentrifuged as before. The final supernatant (particulate fraction) was stored at –70°C. Aliquots of both fractions were used in protein assay by the Bradford method *(11)*.

Alkaline Phosphatase Activity (ALP)

The ALP activity from bovine intestinal mucosa was determined as described by Crans *(12)*. In brief, p-nitrophenylphosphate (pNPP) was used as a substrate in 20 mM Hepes buffer, pH 8.0, in the presence of 20 mM KCl and 30 mM $MgCl_2$. Incubation was carried out at room temperature for 10 min and absorbance at 405 nm was measured in order to determine the initial rate of the activity. Under these experimental conditions, the reaction was linear until 30 min.

The initial rate of ALP activity in the soluble and particulate fractions of UMR.106 cells was determined by incubation of 100 µg protein in 20 mM Hepes buffer pH 8.0/20 mM KCl/30 mM $MgCl_2$ and 5 mM pNPP. Absorbance at 405 nm was monitored at 37°C during 5 min. Under these conditions, the reaction proceeded linearly for 30 min.

The effect of different vanadium compounds and other agents were tested in coincubations with the different enzymes. The inhibitory effect was determined as a percentage of the basal initial velocity of the ALP activity.

Statistical Analysis

The results are expressed as mean ± SEM (n = number of cases) and compared using Student's t-test.

RESULTS

Effect on Bovine Intestinal ALP Activity

The dependence of the rate of the ALP activity from bovine intestine upon vanadate and vanadyl was first investigated. This enzyme had

already been proved to be sensitive to vanadium by Crans et al. *(12)* and was used in these studies as a model. Figure 1 shows the dose-dependent effect of these compounds. Both forms of vanadium were effective inhibitors of ALP activity.

Peroxo and hydroperoxo vanadium compounds were also investigated as to their ability to inhibit ALP. However, the enzyme was not affected at higher concentrations (100 μM) of these vanadium species (Fig. 2).

Effects on UMR.106 Cell ALP Activity

The effects of vanadate and vanadyl ions on the initial rate of soluble and particulate ALP activity of UMR.106 osteoblasts is shown in Fig. 3. As can be seen, inhibition of the soluble enzyme was of a dose-response type (Fig. 3A). The ED50 values show that vanadate seems to be a more effective inhibitor than vanadyl. Particulate activity was inhibited to a lesser extent than that of the soluble form, and it was not of a dose-dependent type. Maximal inhibition was obtained at about 5–10 μM for both vanadium species (Fig. 3B).

Peroxo and hydroperoxo vanadium compounds effectively inhibited soluble ALP activity to the same extent as vanadate. Under similar conditions, particulate ALP activity was not affected by these compounds (Fig. 4).

Partial Characterization of ALP Activity from Osteoblasts

In order to characterize ALP activity from osteoblasts UMR.106, we used a series of compounds already demonstrated to be specific inhibitors of PTPases *(8,10)*. As shown in Table 1, ammonium molibdate inhibits soluble and particulate ALP activity. The inhibition was about 30–40 and 10–20%, respectively. The extent of such effect was similar to that of vanadate. Zn sulfate inhibited about 20% of the basal activity of the soluble ALP. The particulate-associated ALP activity was not inhibited but greatly enhanced by Zn^{2+} (Table 1).

DISCUSSION

There has been an increasing interest in the biological role of vanadium compounds. Vanadium and its derivatives are capable of interacting with various enzyme activities *(3)*. Crans et al. have been working with vanadate and vanadyl on alkaline phosphatase activity. They have found that vanadium interacts with the enzyme, inducing strong inhibitions. Other vanadium derivatives, pervanadates, are also potential inhibitors, as it was previously suggested (4). In this study we did confirm the results of Crans on bovine intestinal mucosa ALP and extended the investigations on peroxo vanadium species. The experiments were

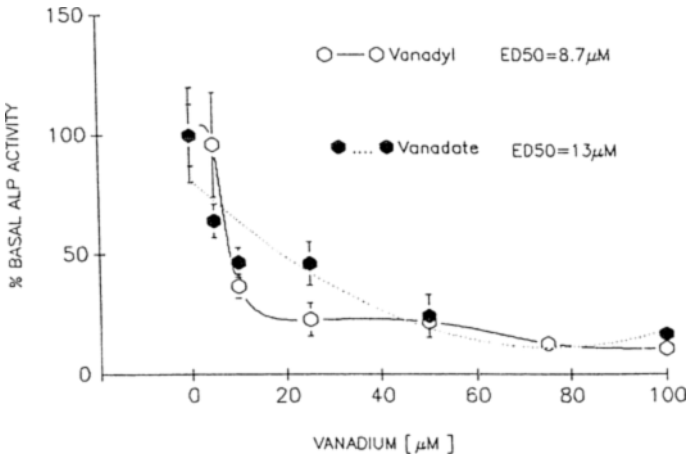

Fig. 1. Effect of vanadate and vanadyl on ALP activity from bovine intestinal mucosa. Initial rate was determined by incubation of the enzyme at 22°C for 10 min in the absence or presence of variable concentrations of the inhibitors. Basal activity was 0.769 ± 0.125 nmol/min/μg protein (n = 3).

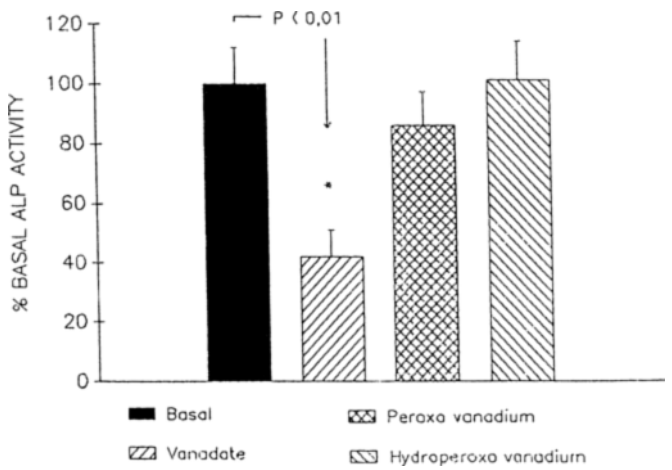

Fig. 2. Effect of different vanadium species on intestinal ALP activity. The initial rate of reaction was measured at 22°C for 10 min without (basal) or with 100 μM vanadium compounds. The basal activity was 0.871 ± 0.094 nmol/min/μg protein (n = 3).

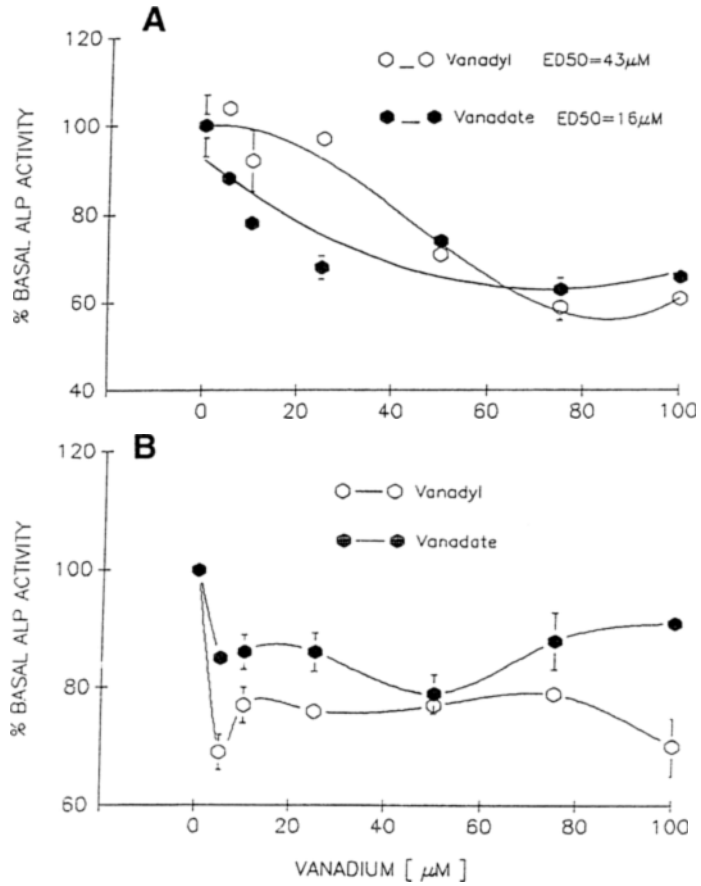

Fig. 3. Effect of vanadate and vanadyl on soluble **(A)** and particulate **(B)** ALP activity from UMR.106 cells. The basal activities were 0.0262 ± 0.002 and 0.0618 ± 0.002 nmol/min/μg protein, respectively (n = 6).

carried out to compare intestinal ALP with enzymes from UMR.106 osteoblasts and to understand the specificity of the different vanadium species as well as their roles in the physiology of the bone.

Vanadate and vanadyl inhibited both soluble and particulate ALP activity from UMR.106 cells and bovine intestinal ALP. Vanadate has been extensively used as specific Tyr-phosphatase inhibitor in different systems (*3*), but Ser/Thr-phosphatases are not sensitive to these compounds (*13*). Vanadium compounds only partially inhibited ALP activity in fraction preparations from UMR osteoblasts, suggesting that Ser/Thr phosphatases, acting at pH 8.0, are also present.

It has been suggested that vanadate may be reduced to vanadyl inside the cells and the latter species seems to be the actual inhibitor (*4*). However, in our cell-free preparations, the two forms were shown to

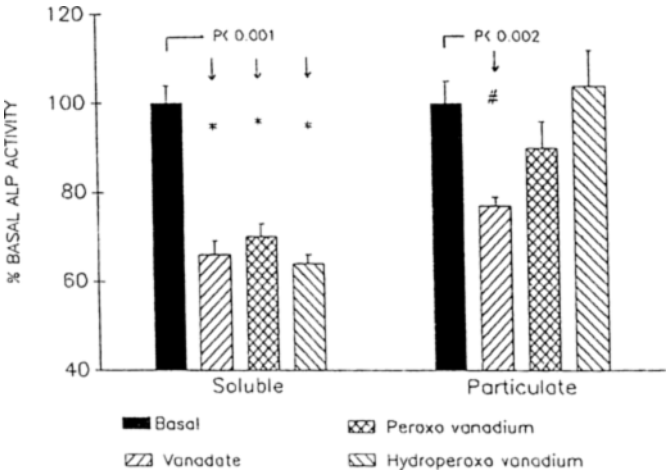

Fig. 4. Effect of different vanadium species on soluble and particulate ALP activity from UMR.106 cells. The concentration of the different compounds was 100 μM and the basal activities were 0.0273 ± 0.001 and 0.0202 ± 0.002 nmol/min/μg protein (n = 6) for soluble and particulate fractions, respectively.

directly interact with ALP. Furthermore, in the soluble fraction, vanadate shows greater inhibition than vanadyl (Fig. 3A).

Peroxo and hydroperoxo vanadium compounds were shown to be more effective inductors of protein phosphorylation in cultured cells than vanadate (*4*). Trudel et al suggest but do not prove that the mechanism may take place through direct inhibition of Tyr-phosphatases. In our experiments, we have shown inhibition of ALP activity in the soluble fraction of osteoblasts by pervanadate forms. ALP activity in the particulate fraction was not inhibited by these species, nor was bovine intestinal ALP.

In other systems it has been shown that pervanadates seem to be selective for the Tyr-phosphatase investigated (*14*). Fantus et al. working with bovine intestine ALP and rat liver microsomes found greater inhibition of tyrosine dephosphorylation with pervanadate than with vanadate. They have also shown a little inhibition of ALP activity with 10–100 μ*M* pervanadate. In our studies, ALP activity in the particulate fraction behaves in a different manner, because it is not inhibited at all.

The assay we used measures total ALP activity, that is Ser/Thr plus Tyr-phosphatases. Moreover, the major component in the particulate fraction is bone ALP, which is a Zn-metalloenzyme previously characterized in membranes of osteoblasts (*15*). ALP in this fraction fullfilled the criteria of Zn-metalloenzyme because it showed great activation by this ion (Table 1). Additionally, in preparations without β-mercaptoethanol, the

Table 1
Effect of Cations on ALP Activity from UMR.106 Cells[a]

Compound	Conc., μM	Percent basal activity	
		Soluble	Particulate
—	—	100 ± 8	100 ± 7
Vanadate	10	78 ± 1	86 ± 3
	50	74 ± 1[b]	79 ± 1[b]
	100	66 ± 1[c]	91 ± 1
NH_4 molybdate	10	78 ± 1[b]	87 ± 2[c]
	50	73 ± 2[b]	—
	100	61 ± 7[b]	73 ± 1[d]
Zn sulfate	10	96 ± 4	161 ± 24
	50	85 ± 7	—
	100	78 ± 4[b]	242 ± 8[e]

[a]Results are expressed as mean ± SEM, $n = 3$. Differences vs basal are:
[b]$p < 0.05$
[c]$p < 0.02$
[d]$p < 0.01$
[e]$p < 0.001$.

effect of Zn was partially lost (data not shown), suggesting that the -SH groups are important for the role of Zn upon the ALP activity of osteoblasts as it has been shown for other Zn-metalloenzymes (*17*).

In the soluble fraction, approx 40% of the ALP activity was characterized as a Tyr-phosphatase. This part of the activity was also inhibited by vanadate, vanadyl, and pervanadate to the same extent. The remaining ALP activity should be associated with Ser/Thr phosphatases.

In summary, this study demonstrates that different forms of vanadium are direct inhibitors of ALP activity. This effect is dependent on the enzymatic activity investigated and on the origin of the ALP and it can account for the stimulation of tyrosine phosphorylation in several systems. The data presented will serve for future investigation of peroxo vanadium compounds in the phosphorylation/dephosphorylation process associated with cell physiology.

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