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VANADATE INHIBITS 2,3-BISPHOSPHOGLYCERATE DEPENDENT PHOSPHOGLYCERATE MUTASES BUT DOES NOT AFFECT THE 2,3-BISPHOSPHOGLYCERATE INDEPENDENT PHOSPHOGLYCERATE MUTASES

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SUMMARY: There are two types of phosphoglycerate mutases. The 2,3-bisphosphoglycerate dependent phosphoglycerate mutases are inhibited by vanadate. In contrast, the 2,3-bisphosphoglycerate independent mutases are not affected. The effect of vanadate varies with pH, and can be reversed by dilution, EDTA and norepinephrine. The differential effect of vanadate on the two types of phosphoglycerate mutases supplies a novel way to easily differentiate both types of enzymes. In addition, it may contribute to the clarification of the mechanism of action of the 2,3-bisphosphoglycerate independent phosphoglycerate mutases.

INTRODUCTION

Phosphoglycerate mutases (PGM), which catalyze the isomerization of 3-phospho-D-glycerate and 2-phospho-D-glycerate, are of two types, those that require 2,3-bisphospho-D-glycerate as a cofactor (BPG-dependent PGM. EC.2.7.5.3) and those that do not (BPG-independent PGM. EC. 5.4.2.1) (1,2). It has been shown recently that the BPG-independent PGM are not exclusive to the plant kingdom, as was suggested by initial reports. The BPG-independent PGM are also present in monera, protist, fungi, and even in some animals (anthozoa, arachnid, echinoderm) (3). The phylogenetic distribution of the two types of PGM has considerable interest from the perspective of biochemical evolution.

Each type of PGM has a different mechanism of action. The BPG-dependent PGM catalyze the intermolecular transfer of phosphoryl groups among the two substrates and the cofactor. The BPG-independent PGM catalyze an intramolecular transfer of the phosphoryl group. However, despite this difference,

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the mechanisms of the two types of PGM have been thought to involve the formation of covalent phosphorylenzymes as intermediates (4-11).

It has been shown that vanadate inhibits a variety of kinases and phosphatases, the mechanisms of which frequently involve a phosphorylenzyme. It has been suggested that pentavalent vanadium can easily adopt a stable trigonal bipyramidal structure, which resembles the transition state of the phosphoryl group (12,13).

This paper shows that the BPG-dependent PGM are strongly inhibited by vanadate. In contrast, the BPG-independent PGM are not affected. This difference permits differentiating easily the BPG-dependent and the BPG-independent PGM. In addition, it may help in clarifying the mechanism of action of the BPG-independent PGM.

MATERIALS AND METHODS

Crystalline PGM and enolase from rabbit muscle, and 2,3-bisphosphoglycerate (pentacyclohexylammonium salt) were from Boehringer Mannheim. 3-phosphoglycerate (barium salt) was from Sigma. Anhydrous sodium metavanadate ($NaVO_3$) was from Merck Darmstadt. L-norepinephrine (L-hydrogen tartrate) was from Fluka. Bovine serum albumin was from Calbiochem. All other chemicals were reagent grade. Sephadex G-100 and DEAE Sephadex A-50 were from Pharmacia. Dowex AG1-X8 (chloride form) was from Bio Rad. Lyophilized preparations of human fibroblasts, ascites tumor cells, neuroblastoma mouse cells (Neuro-2a) and human larynx carcinoma cells (HP-2) were a gift of Drs. C. Guerri and R. Montero.

PGM activity was assayed at 30°C either by coupling the formation of 2-phosphoglycerate from 3-phosphoglycerate with the enolase-catalyzed reaction (14) or by further coupling with pyruvate kinase and lactate dehydrogenase (7). Protein was determined by the methods of Mokrash and McGilvery (15) and Sedmak and Grossberg (16), using bovine serum albumin as a standard. 3-phosphoglycerate free from 2,3-bisphosphoglycerate was prepared by purification on ion-exchange columns (1).

The crystalline rabbit muscle PGM was centrifuged before use and dissolved in 50 mM Tris-HCl buffer pH 7.4 containing 1% bovine serum albumin. The three PGM isozymes from pig heart muscle were partially purified by gel filtration and by ion-exchange chromatography (17). PGM from chicken breast muscle, from yeast and from wheat germ were purified as described up to step II (18,14,3). Extracts from invertebrates were prepared by homogenization on 50 mM Tris-HCl buffer pH 7.4, and ammonium sulphate fractionation (80% saturation) (3). The lyophilized cells were extracted with 50 mM Tris-HCl buffer pH 7.4.

RESULTS

Inhibition of BPG-dependent PGM by vanadate. The effect of vanadate on the activity of the BPG-dependent and BPG-independent PGM from different sources

TABLE I. EFFECT OF VANADATE ON THE ACTIVITY OF THE BPG-DEPENDENT AND THE BPG-INDEPENDENT PHOSPHOGLYCERATE MUTASES

Preparation	% of initial activity	
	10 μM $NaVO_3$	100 μM $NaVO_3$
BPG-dependent PGM		
Yeast	25	5
Rabbit muscle	17	3
Pig heart		
Fast isozyme	20	3
Intermediate isozyme	29	4
Slow isozyme	24	2
Chicken muscle	16	3
Human fibroblasts	12	2
HP-2 epithelial cells	17	1
Ascites tumor cells	16	1
Mouse neuroblastoma	10	3
BPG-independent PGM		
Wheat germ	100	100
Spider	100	100
Haverstmen	100	100
Scorpion	100	100
Centipede	100	100
Sea-urchin (gonad)	100	100

Preparations were obtained as described under "Materials and Methods." The BPG-dependent PGM activity was assayed with the enolase-coupled method, and the BPG-independent activity with further coupling with pyruvate kinase and lactate dehydrogenase. The pH was 7.4 and the temperature 30°C. $NaVO_3$ was added to the assay mixture, and the residual activity measured.

is summarized in Table I. As shown, vanadate inhibited the BPG-dependent activity of the PGM purified from rabbit muscle, the three partially purified PGM isozymes present in pig heart, and the preparations from yeast and from several animal tissues, including tumor cells. When vanadate was added to the assay mixture, inhibition did not occur immediately, but required a few minutes before becoming fully effective. However, when PGM was preincubated with vanadate and the reaction was started by addition of 2,3-bisphosphoglycerate, the reaction rate had the same value as that attained after a few minutes when

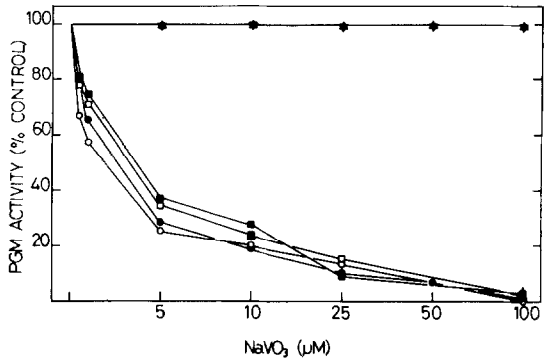

Fig. 1. Effect of vanadate concentration on the activity of the BPG-dependent and BPG-independent phosphoglycerate mutases. PGM activity was assayed at pH 7.4 and at 30°C as stated in Table I in the presence of varying concentrations of $NaVO_3$. Rabbit muscle PGM (●), fast PGM isozyme from pig heart (○), intermediate isozyme from pig heart (■), slow isozyme from pig heart (□), yeast PGM (▲), wheat germ PGM (▼), haverstmen PGM (△).

vanadate was added to the assay mixture after initiation of measurements. The degree of inhibition of several BPG-dependent PGM as a function of vanadate concentration is illustrated in Fig. 1. All PGM showed a similar pattern; 5 μM vanadate was sufficient to cause 50% inhibition, and 100 μM vanadate produced almost complete inhibition.

Vanadate did not affect the BPG-independent PGM activity of extracts from wheat germ or from invertebrates. To eliminate any possible artefact such as

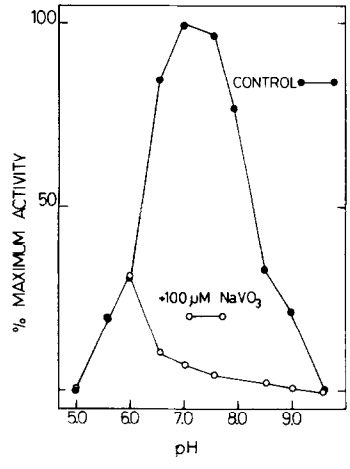

Fig. 2. Effect of pH on the inhibition of rabbit muscle phosphoglycerate mutase by vanadate. PGM mutase activity was assayed at 30°C with the enolase-coupled method. Buffers were 30 mM; acetic acid-sodium acetate (pH 5.0, 5.5, 6.0), imidazole-HCl (pH 6.5, 7.0, 7.5), Tris-HCl (pH 8.0, 8.5, 9.0), sodium bicarbonate-sodium carbonate (pH 10.0). Vanadate ($NaVO_3$) 100 μM was added to the assay mixture, and the residual activity was measured.

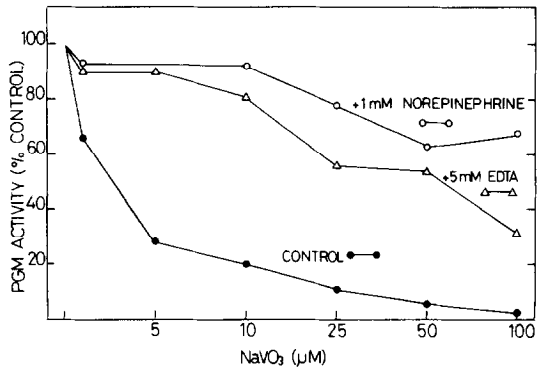

Fig. 3. Preservation by norepinephrine and by EDTA of the inhibition of rabbit muscle phosphoglycerate mutase by vanadate. PGM activity was assayed at pH 7.4 and at 30°C by the enolase-coupled method. Reaction was initiated by adding enzyme to the assay mixture containing either norepinephrine or EDTA. $NaVO_3$ was added at varying concentrations, and the residual activity was measured.

unspecific binding or reduction of vanadium, the effect of vanadate on mixtures of BPG-dependent and BPG-independent PGM was tested. In all experiments, the calculated activity of the BPG-dependent PGM was inhibited, while the mixture showed the expected activity for the BPG-independent enzyme added.

Dependence on pH. The degree of inhibition of the BPG-dependent PGM from rabbit muscle by vanadate varied with pH, as is shown in Fig. 2. Vanadate added to the assay mixture did not affect PGM activity below pH 6.5, and thus yielded, under such conditions, an apparent shift in pH optimum from 7.4 to 6.0.

Reversion by dilution, by EDTA and by norepinephrine. The inhibition of rabbit muscle PGM by vanadate could be reversed by dilution of the inhibited assay mixture with additional fresh assay solution, so that the concentration of vanadate was decreased. As expected, the inhibition caused by vanadate (10^{-4} M) was also reversed by EDTA (5 mM) and by norepinephrine (1 mM). In addition to reversing the inhibition, EDTA and norepinephrine also prevented vanadate inhibition, as shown in Fig. 3.

DISCUSSION

Interest in the biological actions of vanadate has increased greatly in recent years as a result of the fortuitous discovery that some commercial ATP was

contaminated with vanadium and had a marked inhibitory effect on Na^+,K^+ATPase (12). Other enzymes known to be affected by vanadate are some phosphatases and kinases, the mechanisms of action of which frequently involve a phosphorylenzyme intermediate. It has been suggested that vanadate competes with phosphate for binding, because it can easily adopt a stable trigonal bipyramidal structure which resembles the transition state of phosphate during the raction (12,13).

The results presented in this paper demonstrate that BPG-dependent enzymes are markedly inhibited by vanadate, while the BPG-independent PGM are not affected. The inhibition of the BPG-dependent PGM by vanadate varies with pH in a way similar to that observed with the plasma membrane ATPase (19). As has been described with other enzymes, the inhibition can be reversed by decreasing the concentration of free vanadate by dilution. Reversion can be also accomplished by chelation with EDTA and more efficiently by norepinephrine through complexing and reduction of vanadate (20).

It is known that each type of PGM has a different mechanism of action. The BPG-dependent PGM catalyze the intermolecular transfer of the phosphoryl group among the monophosphoglycerates and the 2,3-bisphosphoglycerate. In contrast, the BPG-independent PGM catalyze the intramolecular transfer of the phosphoryl between the two hydroxyl groups of the monophosphoglycerates. Nevertheless, it is believed that there are fundamental similarities in the pathways followed by the two types of PGM, and the mechanism of both types of enzymes appears to involve the formation of a phosphorylenzyme as an intermediate (4-11). In the case of the BPG-dependent PGM, it has been shown that the phosphoryl group is attached to a histidyl residue (4,7,10). In the case of the BPG-independent enzymes, no phosphorylenzyme has been detected but it has been postulated on the basis of indirect evidence (5,11). In view of the findings presented here, further clarification of the mechanism of this unusual type of mutase may be approached anew. The BPG-dependent and independent PGM have been distinguished so far by their requirement for cofactor and by their pH optima (1-3) . Their different responses to vanadate supplies an additional tool to easily differentiate them.

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