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EFFECT OF VANADATE ON PHOSPHORYL TRANSFER ENZYMES INVOLVED IN GLUCOSE METABOLISM

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SUMMARY : Different types of enzymes from yeast and from rabbit muscle which catalyze phosphoryl transfer reactions involved in glucose metabolism differ in their sensitivity to vanadate. Phospho glucomutase and phosphoglycerate mutase are inhibited at the μM range. 2,3-Bisphosphoglycerate phosphatase is completely inhibited by 0.5 mM vanadate. 2,3-Bisphosphoglycerate synthase, hexokinase, phosphoglycerate kinase and fructose-1,6-P_2 phosphatase are partially inhibited by mM vanadate. Phosphofructokinase and pyruvate kinase are not affected. The glycolytic enzymes which mechanism does not involves phosphoryl transfer step are not affected by vanadate.

INTRODUCTION

In recent years it has been shown that vanadium can act as a potent inhibitor of several enzymes which catalyze phosphoryl transfer or release reactions (1, 2) , and it has been suggested that inhibition results because vanadium ions through hydration or chelation can easily adopt a stable trigonal bipyramidal structure which resembles the transition state of phosphate during the reaction (2-5).

We have demonstrated that metavanadate inhibits the 2,3-bisphospho glycerate dependent phosphoglycerate mutase but does not affect the cofactor independent phosphoglycerate mutase (6). This paper shows that different types of enzymes which catalyze phosphoryl transfer reactions involved in glucose metabolism - mutases, kinases and phosphatases - differ in their sensitivity to vanadate. Thus vanadate is a useful tool to investigate the mechanism of the phosphoryl transfer enzymes.

MATERIALS AND METHODS

Rabbit muscle phosphoglucomutase, phosphoglycerate mutase, phospho fructokinase, pyruvate kinase, glyceraldehyde-3-P dehydrogenase, glycerolphosphate dehydrogenase, lactate dehydrogenase, aldolase and enolase, and yeast hexokinase, phosphoglycerate kinase, glucose-6-P

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dehydrogenase, phosphoglucose isomerase and triosephosphate isomerase were from Boehringer Mannheim. Yeast fructose-1,6-P_2 phosphatase was a gift from Dr. C. Gancedo. Substrates and cofactors were from either Boehringer Mannheim or Sigma. Anhydrous sodium metavanadate ($NaVO_3$) was from Merck Darmstad. L-norepinephrine (L-hydrogen tartrate) was from Fluka. Sephadex G-100 and DEAE Sephadex A-50 were from Pharmacia. All other chemicals were reagent grade.

2,3-Bisphosphoglycerate phosphatase and 2,3-bisphosphoglycerate synthase (7,8) were partially purified from rabbit muscle. The fresh tissue was homogenized on 20 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA and 1 mM β-mercaptoethanol, and the extract was precipitated with ammonium sulfate (50-70 % saturation), resuspended and dialyzed against extracting buffer. To obtain the 2,3-bisphosphoglycerate phosphatase, the dialysate was applied to a column of Sephadex G-75 and eluted with buffer. Fractions with 2,3-bisphospho glycerate phosphatase activity not stimulable by glycolate-2-P were pooled (8). To obtain the 2,3-bisphosphoglycerate synthase, the dialysate was applied to a column of DEAE Sephadex A-50 equilibrated with extracting buffer. Phosphoglycerate mutase was washed off and 2,3-bisphosphoglycerate synthase was eluted with a linear gradient of NaCl (7,8).

Hexokinase was extracted from rabbit muscle by homogenization on 10 mM K-phosphate buffer (pH 7.0) containing 5 mM β-mercaptoethanol, 5 mM EDTA and 10 mM glucose (9). After precipitation with 50 % ammonium sulfate, the sediment was dissolved and dialyzed against extracting buffer.

Phosphoglycerate kinase was extracted from rabbit muscle with 50 mM Tris-HCl buffer pH 7.5, precipitated with 75 % ammonium sulfate and dialyzed against the same buffer.

Phosphoglycerate mutase and phosphoglucomutase were partially purified from yeast, following the procedure described by De la Morena et.al. up to step II (10).

Phosphoglucomutase was assayed in the presence of 2 mM glucose-1-P, 20 μM glucose-1,6-P_2, 0.5 mM NADP, 10 mM $MgCl_2$, 40 mM triethanolamine buffer (pH 7.6) and 1 U/ml of glucose-6-P dehydrogenase. The rabbit muscle enzyme was preincubated for 10 minutes with 32 mM histidine before assay (11). Phosphoglycerate mutase was tested by the enolase-coupled method (10) in the presence of 20 mM glycerate-3-P, 0.25 mM glycerate-2,3-P_2, 3 mM $MgSO_4$, 32 mM Tris-HCl buffer (pH 7.5) and 1 U/ml of enolase. Yeast and rabbit muscle hexokinase were tested according (12) and (9), respectively. Phosphofructokinase, phosphoglycerate kinase and pyruvate kinase were assayed following (12). 2,3-Bisphosphoglycerate synthase was determined by the "Method A" described by Joyce and Grisolia (13). The glycerate-2,3-P_2 synthesized was stimated by the "Method 2" of Towne et. al. (14). When the sample to be assayed contained vanadate, 5 mM EDTA was added to the assay mixture (6). 2,3-Bisphosphoglycerate phosphatase was assayed as described (15), determining the released Pi by the method of Gomori (16). Fructose-1,6-P_2 phosphatase was assayed according to (17). Glucose-6-P dehydrogenase, glycerolphosphate dehydrogenase, lactate dehydrogenase, phosphoglucose isomerase, triosephosphate isomerase and aldolase were determined according to (12). Phosphoglyceraldehyde dehydrogenase and enolase were tested following to (18) and (19), respectively. All measurements were performed at 30°C.

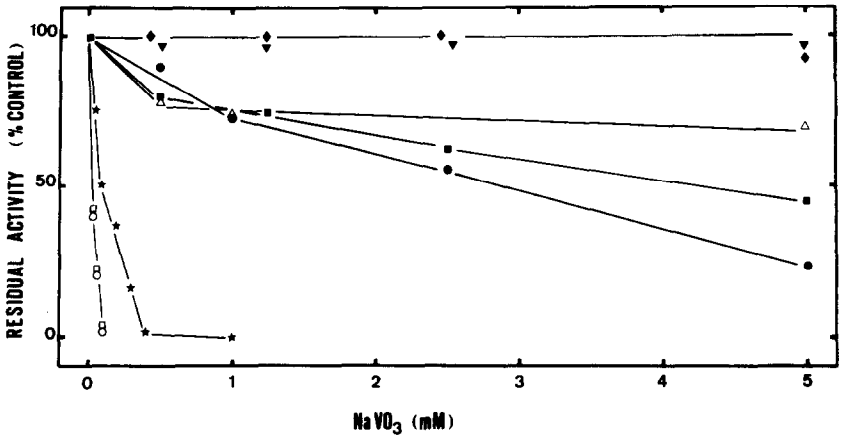

Fig.1 - Effect of vanadate on the activity of rabbit muscle enzymes
Enzymatic activities were assayed as stated under "Materials and Methods" in the presence of varying concentrations of $NaVO_3$. Phospho glucomutase (O), phosphoglycerate mutase (□), 2,3-bisphospho-glycerate synthase (△), 2,3-bisphosphoglycerate phosphatase (✱), hexokinase (●), phosphoglycerate kinase (■), pyruvate kinase (▼), phosphofructokinase (◆).

The inhibitory effect of vanadate was tested both by addition of metavanadate to the assay mixture and by preincubation of the enzyme with vanadate before starting reaction. Control experiments were done to discard inhibition of the coupled enzymes by vanadate. Sodium vanadate was dissolved in water and used within 15-20 hours.

RESULTS

As shown in Figs. 1 and 2, metavanadate markedly inhibited phospho glucomutase and phosphoglycerate mutase from yeast and from rabbit

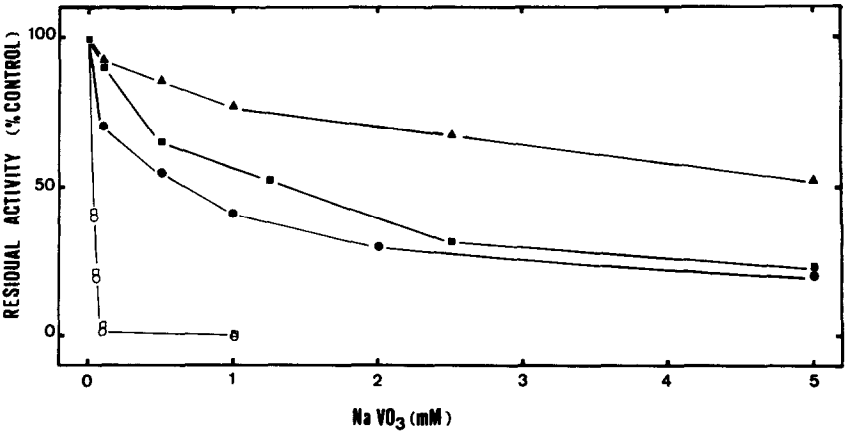

Fig.2 - Effect of vanadate on the activity of yeast enzymes
Phosphoglucomutase (O), phosphoglycerate mutase (□), hexokinase (●), phosphoglycerate kinase (■), fructose-1,6-P_2 phosphatase (▲).

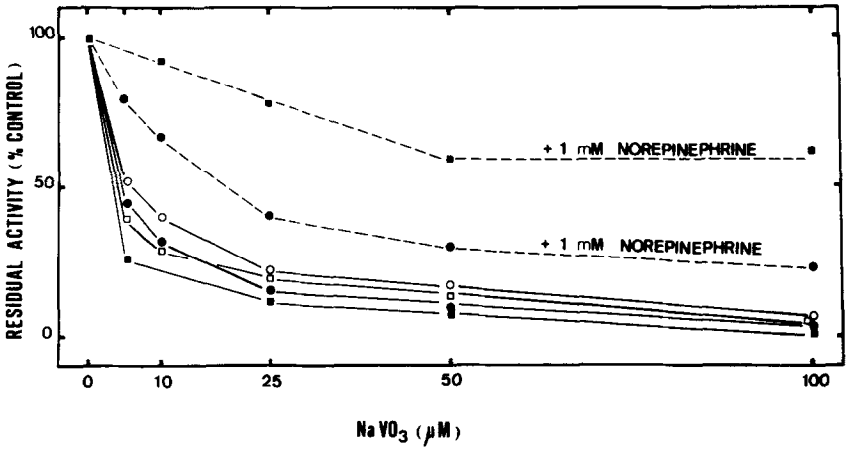

Fig.3 - Effect of vanadate on the activity of phosphoglucomutase and phosphoglycerate mutase from rabbit muscle and from yeast
Reaction was initiated by adding enzyme to the assay mixture containing or not 1 mM norepinephrine. $NaVO_3$ was added at varying concentrations, and the residual activity was measured. Rabbit muscle phosphoglucomutase (●), yeast phosphoglucomutase (○), rabbit muscle phosphoglycerate mutase (■), yeast phosphoglycerate mutase (□).

muscle. When vanadate was added to the assay mixtures, inhibition required a few minutes before becoming fully effective. When the enzymes were preincubated for a few minutes with vanadate and the reaction was started by addition of the cofactor, the inhibition was instant from the beginning. Both mutases showed a similar inhibitory pattern, being almost completely inhibited by 100 μM vanadate. The inhibitory effect could be reversed by dilution of the inhibited assay mixture with fresh assay solution. Inhibition was also reversed by 1 mM norepinephrine, as shown in Fig. 3.

2,3-Bisphosphoglycerate phosphatase partially purified from rabbit muscle was also inhibited by vanadate, although inhibition was not as high as that observed with phosphoglucomutase and phosphoglycerate mutase. As shown in Fig. 1, the activity was reduced 50 % by 0.1 mM vanadate and was almost completely abolished by 0.4 mM $NaVO_3$.

2,3-Bisphosphoglycerate synthase from muscle, hexokinase and phosphoglycerate kinase from muscle and from yeast, and fructose-1,6-P_2 phosphatase from yeast were partially inhibited by vanadate. As shown in Figs. 1 and 2, activity was present even with the larger concentration of vanadate tested (5 mM).

Phosphofructokinase and pyruvate kinase from muscle were the only phosphoryl transfer enzymes not affected by vanadate. They retained

the total activity even in the presence of 5 mM vanadate, as shown in Fig. 1.

Glucose-6-P dehydrogenase, glycerolphosphate dehydrogenase, phosphoglyceraldehyde dehydrogenase, lactate dehydrogenase, phosphoglucose isomerase, triosephosphate isomerase, aldolase and enolase were resistent to vanadate.

DISCUSSION

The elementary steps of the enzyme-catalyzed phosphoryl transfer or release reactions may proceed through either a dissociative or an associative pathway. The dissociative displacement involves the transient formation of the intermediate monomeric metaphosphate, and the associative mechanism involves either a pentavalent trigonal bipyramidal transient state or a pentacoordinate covalent intermediate (20). The binding of vanadium ions to ribonuclease and to some phosphatases with concomitant inhibition of the enzymatic activity suggested that vanadium compounds act as structural and electronic analogues of the transition states for associative displacements. It has been postulated that oxovanadium (IV) and vanadium (V) ions are highly effective as inhibitors because through hydration or chelation can easily adopt a stable trigonal bipyramidal structure which resembles the transition state of phosphate during reaction (3-5). On this basis the possibility that vanadium effects might help to distinguish between associative and dissociative mechanisms have to be considered (20).

Of the phosphoryl transfer enzymes from yeast and from rabbit muscle studied by us, phosphoglucomutase and phosphoglycerate mutase were the only enzymes markedly inhibited by vanadate at the μM range. Both mutases require the bisphosphosubstrate as a cofactor, and catalyze the intermolecular transfer of the phosphoryl group between the two monophosphorylated substrates and the bisphosphorylated cofactor by a mechanism which involves a phosphorylenzyme as an intermediate (20). It has been reported that orthovanadate activates the transfer of ^{32}P from [^{32}P] phosphoglucomutase to glucose, possibly by interaction with a tyrosine residue at the active site (21).

2,3-Bisphosphoglycerate synthase is an enzyme closely related to phosphoglycerate mutase which also proceeds by a phosphorylenzyme pathway (22). In contrast with the mutases, 2,3-bisphosphoglycerate synthase from muscle is only partially inhibited by metavanadate even at the mM range.

Most phosphokinases follow a sequential pathway in which phosphoryl transfer seems to occur directly between the substrate molecules within the ternary complex without the covalent intervention of the enzyme. The experiments done in an effort to distinguish between an associative and a dissociative mechanism have not been conclusive (20). We have found that mM vanadate partially inhibits hexokinase and phosphoglycerate kinase, and does not affect phosphofructokinase or pyruvate kinase. The lack of inhibition of phosphofructokinase by metavanadate has been reported by Choate and Mansour, who have found that decavanadate inhibits the enzyme in a manner similar to the allosteric inhibition by ATP (23).

The phosphatases - ATPases, alkaline phosphatase and acid phosphatase - which have been found to be inhibited by vanadate (2,4,5) proceed by a phosphorylenzyme mechanism (20). We have found that yeast fructose-1,6-P_2 phosphatase, for which a sequential mechanism has been postulated (17), is only partially affected by mM vanadate. A similar degree of inhibition has been reported for liver glucose-6-P phosphatase (24). 2,3-Bisphosphoglycerate phosphatase free from phosphoglycerate mutase and 2,3-bisphosphoglycerate synthase activities, which has been recently found in muscle (7,8), is more sensitive to vanadate. Its activity is almost completely inhibited by 0.5 mM vanadate.

On basis of the effects of vanadium on the activity of several enzymes, it has been postulated that vanadium may have a regulatory role (1,2). This study shows that metavanadate at physiological concentrations does not affect the key enzymes of the glycolytic pathway.

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