VANADATE INHIBITS MEVALONATE SYNTHESIS AND ACTIVATES NADH OXIDATION IN MICROSOMES

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1. Introduction

Vanadate gained prominence after its powerful inhibitory effect on Na,K-ATPase was discovered [1–4]. Dynein ATPase, myosin ATPase, red cell Ca^{2+}-ATPase, acid phosphatase, alkaline phosphatase, monoamine oxidase, succinate dehydrogenase, adenylate kinase, phosphofructokinase and ribonuclease are known to be inhibited by vanadium compounds (reviewed [5]). Vanadate is reported to decrease plasma cholesterol and inhibit cholesterol biosynthesis and this effect is ascribed to a possible inhibition of squalene oxidase [6]. We now report that vanadate inhibits microsomal 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis in the liver and also its inactivating enzyme. It was found that oxidation of NAD(P)H was enhanced considerably by vanadate while checking whether the reducing component in the reductase reaction, NADPH, was depleted, but this was not responsible for the inhibition of the reductase.

2. Materials and methods

3-Hydroxy-3-[1-^{14}C]methylglutarate (7.7 mCi/mmol) was obtained from New England Nuclear, Boston, MA and the substrate HMGCoA was synthesized as in [7]. NADH, ATP, G-6-P, G-6-P dehydrogenase and NADPH were obtained from Sigma Chemical Co., St Louis, MO. All other reagents were of analytical grade.

Male albino rats (100–120 g) obtained from the Institute colony were killed at midnight by cervical dislocation, the excised livers were homogenized in potassium phosphate buffer (pH 7.4, 50 mM) containing EDTA (30 mM), NaCl (70 mM) and dithiothreitol (DTT, 10 mM). Microsomes were prepared from these homogenates and HMGCoA reductase was assayed in presence of NADPH-regenerating system as in [8]. For assaying HMGCoA reductase inactivating enzyme, the reaction mixture for HMGCoA reductase was first preincubated with ATP · Mg (1 mM) at 37°C for 10 min to inactivate the reductase and then the assay for the reductase was carried out as above. The reductase activity sensitive to ATP · Mg is taken as a measure of the inactivating enzyme.

NAD(P)H oxidase activity was measured by the uptake of oxygen in a Gilson oxygraph fitted with a Clark electrode [9]. Microsomes for this purpose were isolated from liver homogenates in 0.25 M sucrose.

Vanadate solution was prepared by dissolving V_2O_5 in a known amount of alkali and the resulting neutral solution, yellow in colour, containing a mixture of orthovanadate and decavanadate, was used.

3. Results and discussion

The activity of HMGCoA reductase was inhibited by vanadate over 1–10 mM (fig.1). Added vanadate in the reaction mixture was found to be reduced by the excess DTT present as indicated by the blue–green colour formation. Therefore, it is possible that the active form may be the reduced vanadyl species as in the case of alkaline phosphatase [10] but the answer must await further experiments. We tested whether the inhibition of the reductase was due to the effect of vanadate on any of the other enzymes indirectly connected with the assay.

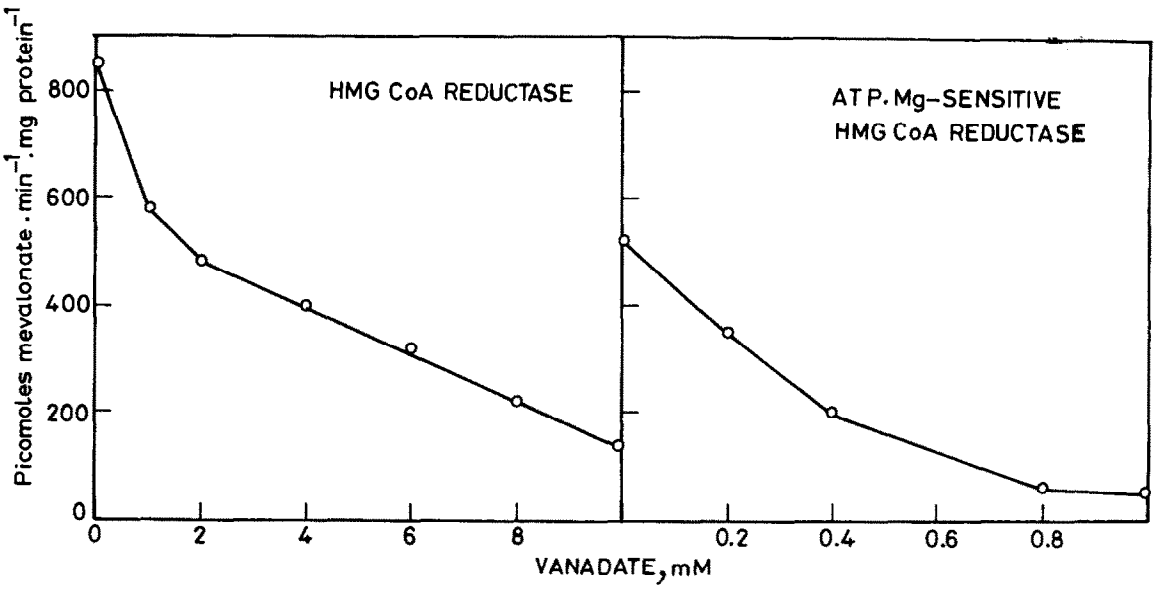

Fig.1. Inhibition of HMGCoA reductase and its sensitivity to ATP · Mg. The reaction mixture for the assay of HMGCoA reductase contained microsomal protein (0.3 mg), [3-^{14}C]HMGCoA (50 nmol, 365 cpm/nmol), NADP (365 nmol), G-6-P (4.5 μmol), G-6-P dehydrogenase (1 unit) in phosphate buffer (50 mM, pH 7.4) containing NaCl (70 mM) and DTT (10 mM). The assay mixture was incubated at 37°C for 20 min, and mevalonate formed was estimated [7]. ATP · Mg-sensitive HMGCoA reductase was assayed by incubating the microsomes with ATP · Mg (1 mM) for 10 min and then assaying for the reductase activity.

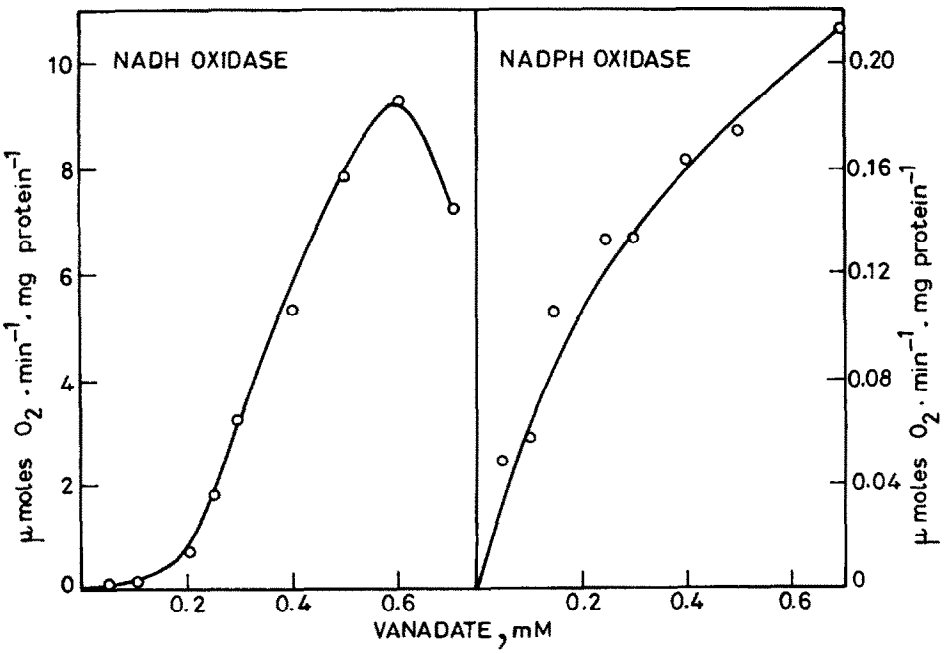

Fig.2. Activation of NAD(P)H oxidase. NAD(P)H oxidase was assayed in microsomes isolated in 0.25 M sucrose. The reaction mixture contained microsomal protein (0.2 mg) NAD(P)H (0.15 mM) in phosphate buffer (0.05 M, pH 7.0) and the uptake of oxygen was measured [10].

The possibility of increased hydrolysis of HMGCoA and thereby depleting the substrate was ruled out as the amount of free [^{14}C]HMG was found to be the same (~9500 cpm) in both control and vanadate samples in the reductase assay mixtures.

NADPH was maintained in the reaction mixture by the regenerating system of G-6-P dehydrogenase. This enzyme was unaffected at the concentrations of vanadate used. The substrate for this enzyme, G-6-P, is hydrolyzed by a phosphatase present in microsomes. Addition of vanadate in fact inhibited this enzyme (~50% at 1 mM) and thus spared G-6-P. Therefore the NADPH-regenerating system remains unaffected in presence of vanadate.

Under the experimental conditions of the reductase assay, NADPH is not oxidized at any appreciable rate. Addition of vanadate showed a concentration-dependent increase in NADPH oxidase (fig.2). In order to ensure NADPH was not depleted, a 2-fold excess of G-6-P dehydrogenase was added in the reaction mixture of the reductase assay. In presence of the highest concentration of vanadate used (10 mM), the A_{340} was found to remain constant until the end of 20 min incubation time, under these conditions.

Microsomes also have negligible NADH oxidase activity and stimulation of this by vanadate was more marked, the rate being 30–40-fold higher than NADPH (fig.2). This activity can be measured by the oxygen uptake as well as by a decrease in A_{340}. A ratio of ~1.0 was obtained for NADH/O_2 and H_2O_2 appears to be the product.

Stimulation of NADH oxidase was not obtained with reduced vanadyl form and also by metavanadate but required vanadate possibly in the polymeric form found in the vanadate solution as prepared. The reported stimulation of NADH oxidation by cat cardiac cell membranes by vanadate may also represent a vanadate-stimulated NADH oxidase instead of NADH-vanadate reductase as suggested [11].

Microsomes have an enzyme that inactivates HMGCoA reductase on preincubation in vitro with ATP · Mg and this is considered to be a kinase of the reductase [12]. This inactivating effect of ATP · Mg was progressively lost when increasing concentrations of vanadate were included in the preincubation mixture (fig.1). Mg-activated ATPase activity in microsomes was completely inhibited at 1 mM vanadate. Therefore, vanadate would have spared ATP and the loss of inactivating effect must be direct and not by depleting ATP. The inactivating enzyme is relatively more sensitive to vanadate, being largely inhibited at 1 mM, whereas the reductase required over 10 mM for complete inhibition. In view of this, cholesterol synthesis should increase at low concentrations of vanadate and decrease at higher concentrations. The variable effects of vanadate on cholesterol synthesis and plasma cholesterol [6] may thus be explained.

It is known that the effects of vanadate are mostly inhibitory, particularly on a number of phosphohydrolases. So far only one other enzyme, adenyl cyclase, is known to be activated by vanadate [13,14]. Our results now show that a NADPH-dependent reductase can be inhibited and NAD(P)H oxidase can be stimulated by vanadate. Thus vanadate may have a role in controlling redox systems at the endomembrane level and these effects may be helpful in explaining the essential nature of this trace element in nutrition and its toxicity in excess.

Acknowledgements

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