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REDUCTION OF VANADATE BY A MICROSOMAL REDOX SYSTEM

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Summary: The reduction of vanadate catalyzed by rat liver microsomes is demonstrated. This reaction is SOD-insensitive. It is specific for NADH and polyvanadate and is not obtained with metavanadate and NADPH.

Addition of vanadate was first shown to increase the rate of disappearance of NADH obtained with cat ventricle membranes and this was ascribed to the presence of NADH-vanadate reductase (1). Polyvanadate-mediated electron transfer from NADH to oxygen with generation of H_2O_2 was demonstrated in mouse liver plasma membranes (2). The reaction occurs only in presence of oxygen, without obvious reduction of vanadate, and is found in variety of endomembranes (3,4). The stoichiometry of NADH disappearance and oxygen uptake was 1:1. The activity increased several fold in acid pH range. Phosphate was needed for maximum activity. Superoxide dismutase, the enzyme known to dismutate O_2^- to H_2O_2, inhibited this activity indicating that superoxide was not an end-product but an intermediate in the reaction (2-4). Vanadate, therefore, acts as an intermediate electron carrier between NADH and oxygen but does not act as an electron sink.

Vanadate in the reduced state occurs in trace quantities in animal and human tissues (5) and as a large accumulation in tunicates (6). Existence of a reducing mechanism for vanadate is implicit. Only one report is available on such a reaction utilizing glutathione in erythrocytes but it is not clear whether it is enzymatic (7). We have reported that addition of vanadate stimulated oxidation of NADH by rat liver microsomes (8). We now show for the first

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time, that vanadate can be reduced by a SOD-insensitive enzymatic reaction by a microsomal redox system.

Experimental

Oxidation of NADH and reduction of polyvanadate were followed by the decrease in A_{340nm} and by the increase in A_{650nm}, respectively, in a double beam Shimadzu recording spectrophotometer. The reaction mixture of 1 ml contained 50 mM phosphate buffer (pH 7.0), NADH (50-200 µM), polyvanadate (100 µM) and microsomal protein (25 µg). Oxygen uptake studies were carried out in Gilson oxygraph fitted with Clark oxygen electrode.

Polyvanadate solution was prepared by extracting overnight solid vanadium pentoxide (V_2O_5) with 0.3 M carbonate-free NaOH. The orange yellow color solution, containing predominantly polyvanadate (9), is taken to be equivalent to 0.1 M vanadate.

Results and Discussion

The rate of vanadate-dependent NADH oxidation by rat liver microsomes was decreased, but not abolished, in presence of SOD or under anaerobic conditions unlike the plasma membrane systems (5). This implied that a component other than oxygen can also act as the electron acceptor with the microsomal system. Among the components in the reaction mixture only vanadate, being a transition element, has this potential.

Formation of 650 nm absorbing compound: A faint blue color could be seen in the reaction mixture of polyvanadate-dependent oxidation of NADH by microsomes. The spectra of this solution recorded at the beginning (spectrum I) and 3-4 min after the addition of microsomes (spectrum II) showed the formation of a species with broad absorbance in the red region with a peak at about 650 nm (Fig.1A). This 650 nm-absorbing compound is likely to be a species of reduced vanadate (V^{iv}), known to be blue in color, but is different from vanadyl sulfate (spectrum III). The 650 nm-absorbing species decreased after reaching a maximum at about 4 min, by which time NADH is oxidized (Fig.1B).

The ratio of rates of oxidation of NADH:NADPH is about 4 (Fig.2A). But there was hardly any reduction of vanadate to the blue colored 650 nm compound with NADPH (Fig.2B). We found that NADH oxidation by microsomes was not supported by metavanadate and also that no vanadate reduction was obtained in the absence of NADH oxidation. These results indicate that

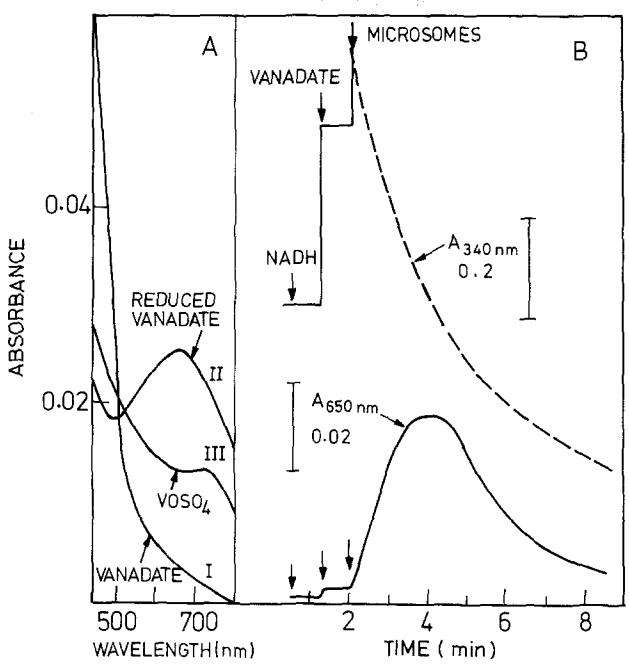

Fig. 1. Absorbance change in red region during NADH oxidation

A. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 50 µM NADH and 100 µM polyvanadate in 1 ml. The spectrum of this solution was initially taken (I), rat liver microsomes (25 µg protein) were then added into both cuvettes and the spectrum was recorded between 3-4 min (II). For comparison spectrum of 1 mM aqueous vanadyl sulphate is given (III).

B. Time study of the decrease in absorbance at 340 nm indicating disappearance of NADH and increase in absorbance of 650 nm showing formation of a reduced vanadate compound in the reaction mixture is given above.

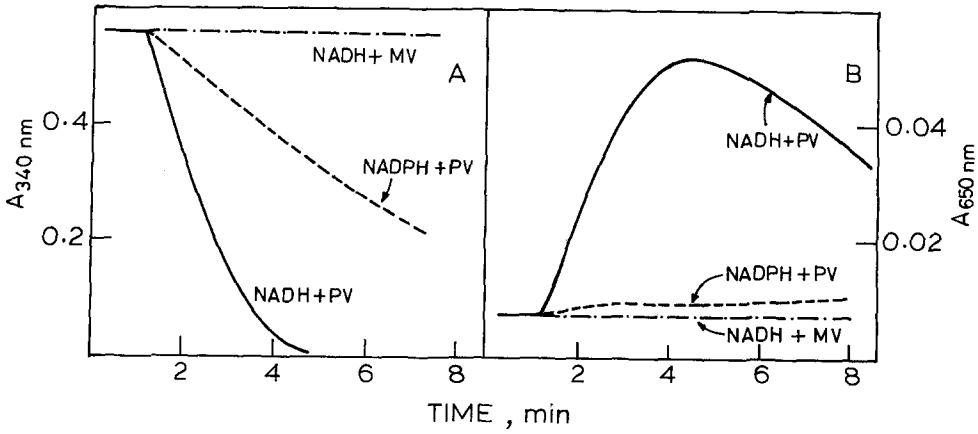

Fig. 2. Requirements for vanadate reduction

A. The standard reaction mixture was used to study NAD(P)H oxidation followed by decrease in $A_{340\,nm}$.

B. Under similar conditions vanadate reduction was followed as seen by increase in $A_{650\,nm}$. The reaction mixture had polyvanadate (PV) or metavanadate (MV) and NADH or NADPH (100 µM), where indicated.

Table 1. Effect of superoxide dismutase on polyvanadate-mediated oxidation of NADH

Measurement	Rate	-SOD	+SOD
NADH disappearance	$A_{340\ nm/min}$	0.17	0.14
Oxygen uptake	n moles O_2/min	22.0	2.2
Polyvanadate reduction	$A_{650\ nm/min}$	0.014	0.015

The reduction mixture consisted of 50 mM phosphate buffer (pH 7.0), NADH (100 µM), polyvanadate (100 µM) and rat liver microsomes (protein 35 µg/ml). When uptake of oxygen was measured with a Clark electrode the reaction mixture contained 200 µM NADH.

reduction of vanadate can only be obtained with polyvanadate and NADH in the microsomal system under study.

Effects of SOD on NADH disappearance and vanadate reduction

Addition of SOD (10 µg/ml), known to abolish polyvanadate-stimulated NADH oxidation in plasma membranes (2-4), had little inhibitory effect on NADH disappearance when rat liver microsomes were used as the enzyme source (Table 1). Under these conditions oxygen uptake was inhibited by 90%, the rate of oxidation of NADH decreased by 24% and there was no change in the rate of formation of 650 nm-absorbing compound. Spectroscopic studies indicated that electron flow to polyvanadate is through cytochrome b_5. These results are consistent with a SOD-insensitive NADH-dependent polyvanadate reduction.

A rapid enzymatic reduction of vanadate of the type described here is essential in all tissues to keep this purported micronutrient in a less toxic state.

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