INHIBITION OF THE RESPIRATORY SYSTEM IN *AZOTOBACTER VINELANDII* BY DIVALENT TRANSITION METAL IONS

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1. Introduction

The obligate aerobe N_2-fixing bacterium *Azotobacter vinelandii* contains an extremely complex respiratory system [1]. Many classical inhibitors of mitochondrial electron transfer such as rotenone or antimycin A do not act on the *Azotobacter* chain. Only 2-*n*-alkyl-4-hydroxyquinoline-*N*-oxide, cyanide and carbon monoxide have thus far been found to be effective. In this report I wish to present data, which show that some divalent transition metal ions, especially Zn^{2+}, are potent inhibitors of the respiratory system in *A. vinelandii*, and that the electron transport scheme in [2] cannot explain the type of inhibition.

2. Materials and methods

Azotobacter vinelandii strain OP was grown under N_2-fixing conditions and harvested in the late logarithmic phase. Small electron transport particles were prepared according to [3] and washed and resuspended in 20 mM Tris–HCl buffer (pH 7.5). They were used either freshly prepared or after storage at −15°C. Although the dehydrogenase systems changed their specific activity upon storage, the inhibition results were only slightly affected. Oxidation of succinate, lactate, malate and ascorbate/dichlorophenol indophenol were followed with an oxygen electrode, oxidation of NADH and NADPH were measured photometrically at 340 nm. All reactions were carried out in 20 mM Tris–HCl (pH 7.5) at 30°C. Protein concentrations were estimated by the biuret method. Fe^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+} and Cd^{2+} were added as sulfates, Co^{2+} and Mn^{2+} as chlorides.

3. Results

Zn^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+} and Co^{2+} inhibit the oxidation of NADH to various degrees (fig.1). 50% inhibition is effected by concentrations between 3 and 30 μM (table 1). Mn^{2+} and Fe^{2+} are ineffective up to 0.8 mM. Oxidation of NADPH, succinate and lactate is also inhibited by those metal ions. Figure 2 gives the data for Zn^{2+}, the most potent inhibitor. Oxidation of malate is only blocked by Cd^{2+} and Cu^{2+} at far higher concentrations (table 1). None of the metal ions has any effect on the oxidation of ascorbate/dichlorophenol indophenol below 1 mM.

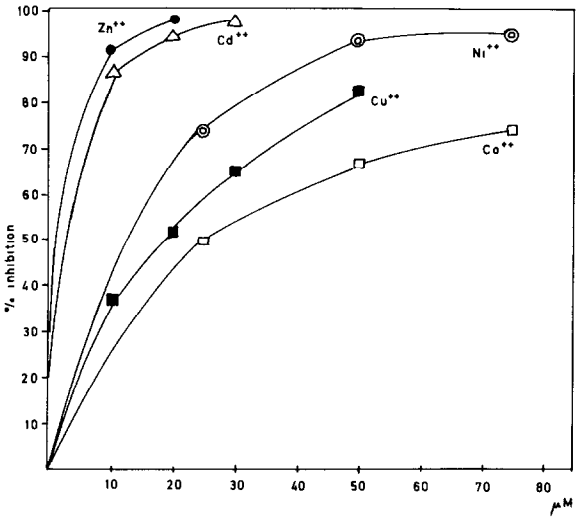

Fig.1. Inhibition by transition metal ions of the NADH oxidation catalyzed by membrane fragments of *Azotobacter vinelandii*. Conditions: 0.065 mg protein in 1 ml 20 mM Tris (pH 7.5), t = 30°C, 0.25 mM NADH.

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Table 1
50% Inhibition of substrate oxidation by indicated metal ion concentration (in μM)

Substrate	Metal ion				
	Zn^{2+}	Cd^{2+}	Cu^{2+}	Ni^{2+}	Co^{2+}
NADH	3	4	20	13	25
NADPH	8	25	40	120	100
Succinate	12	12	35	250	400
Lactate	130	160	130	420	500
Malate	>1000	320	400	>1000	>1000
Ascorbate/dichloro-phenol indophenol	>1000	>1000	>1000	>1000	>1000

The inhibition in all cases can be relieved by EDTA. With succinate as substrate the addition of EDTA to the Me^{2+}-blocked respiratory system produces a higher oxidation rate than that of the uninhibited chain (fig.3a). This enhancement is not due to EDTA alone or the EDTA–Me^{2+} complex (fig.3b).

4. Discussion

Zn^{2+} are known as specific and potent inhibitors of mitochondrial electron transport [4–7]. The site of inhibition is located between ubiquinone and cytochrome *b* [6,7]. Other divalent cations are >100-times less effective with the exception of Cu^{2+}, which does not inhibit at the same site [8]. This work demonstrates that Zn^{2+} in the same concentration range inhibit electron transport in *A. vinelandii*. As oxidation of ascorbate is not inhibited, and as

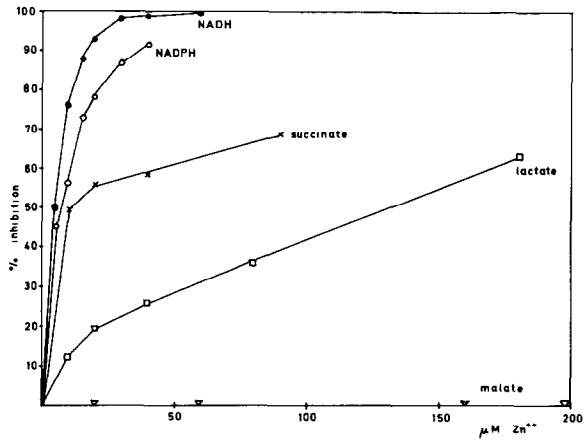

Fig.2. Inhibition by Zn^{2+} of the oxidation of various substrates. Conditions: NADH and NADPH oxidation same as in fig.1; oxidation of other substrates in 5 ml 20 mM Tris (pH 7.5), t = 30°C; concentrations: 10 mM succinate or lactate, 7 mM malate, 3.2 mg protein.

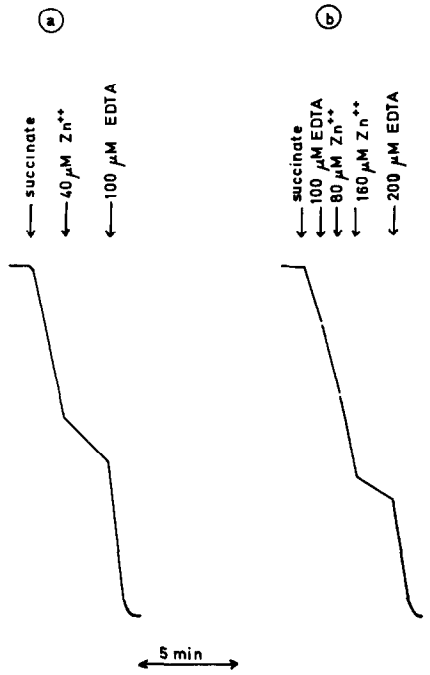

Fig.3. Inhibition of succinate oxidation by Zn^{2+} and reversion by EDTA. Final concentrations are indicated. Other conditions as in fig.2.

oxidation of both NADH and NADPH can be inhibited completely, the same binding site as in mitochondria may be involved, since the dehydrogenases of NADH, NADPH and succinate are supposed to join at ubiquinone, and beyond cytochrome *b* the chain is branched again [9]. The inhibition seems not to be as specific for Zn^{2+} as in mitochondria, since other metal ions are effective too, albeit to a lesser degree. The finding, that malate oxidaton is not inhibited by Zn^{2+}, is not compatible with the concept of the respiratory system proposed in [2]. According to that proposal electrons coming from malate are funnelled into the ubiquinone pool. This problem and the enhancement of succinate oxidation after reversion of the metal ion inhibition by EDTA require further investigations.

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