INHIBITION OF ENERGIZATION OF *SALMONELLA TYPHIMURIUM* MEMBRANE BY ZINC IONS

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1. Introduction

Zn^{2+} inhibits the transport of sugars and amino acids into intact cells of *Escherichia coli* [1] and *Pseudomonas aeruginosa* [2]. Sulfhydryl groups, the likely targets for inhibition by Zn^{2+}, have been reported to be components of the transport system for these substances in bacterial membranes [3]. Thus, it is not possible to determine if Zn^{2+} inhibits at the level of the transport components or whether it prevents energization of the membrane.

To answer this question we have studied the effect of Zn^{2+} on the energy-dependent transhydrogenation of $NADP^+$ by NADH in membrane particles of *Salmonella typhimurium*. This system can be driven by either NADH or ATP [4]. The energized state so formed is probably that which is also involved in the active transport of sugars and amino acids [5]. Effects of inhibitors directly on the transhydrogenase enzyme itself, as opposed to the energization process, can be independently measured by studying their action on the energy-independent transhydrogenase reaction [6,7].

This note reports evidence that Zn^{2+} inhibits oxidation of various substrates and interferes with the energization process.

2. Experimental

Salmonella typhimurium, LT2 was grown in a glucose–salts minimal medium containing 12 μM ferric citrate at 37°C with aeration. Bacteria were harvested in the late log phase of growth and membrane particles were prepared as previously described for *E. coli* [8]. The assays of energy-dependent, energy-independent transhydrogenase, and succinate oxidase were carried out by the methods of Bragg et al. [8], Kaplan [9], and Luzikov and Romashina [10], respectively. NADH oxidase was measured spectrophotometrically in a reaction volume of 1.0 ml containing 50 mM Tris–HCL buffer, pH 7.5, 5 mM $MgCl_2$, 0.15 mM NADH and 100 μg membrane particle protein. The reaction was monitored at 37°C with a Coleman 124 spectrophotometer. D-Lactate and succinate dehydrogenase activities were assayed by the method of Rogers et al. [11]. Ca^{2+}-activated ATPase was measured as previously described [12].

3. Results and discussion

The effect of Zn^{2+} on the energy-dependent (aerobic-driven and ATP-driven) and energy-independent transhydrogenases, and succinate and NADH oxidase activities in membrane particles of *S. typhimurium*, is shown in fig. 1. Zn^{2+} had little effect on the energy-independent transhydrogenase but it effectively inhibited both energy-dependent transhydrogenase reactions (fig. 1A,B). The concentrations of Zn^{2+} used had no effect on the NADH generating system. The aerobic-driven reaction was more sensitive to the inhibitor than the ATP-driven transhydrogenase. The effect of the Zn^{2+} on the former reaction could be correlated with an inhibitory action on NADH oxidation (fig. 1D). Inhibition of NADH oxidase would

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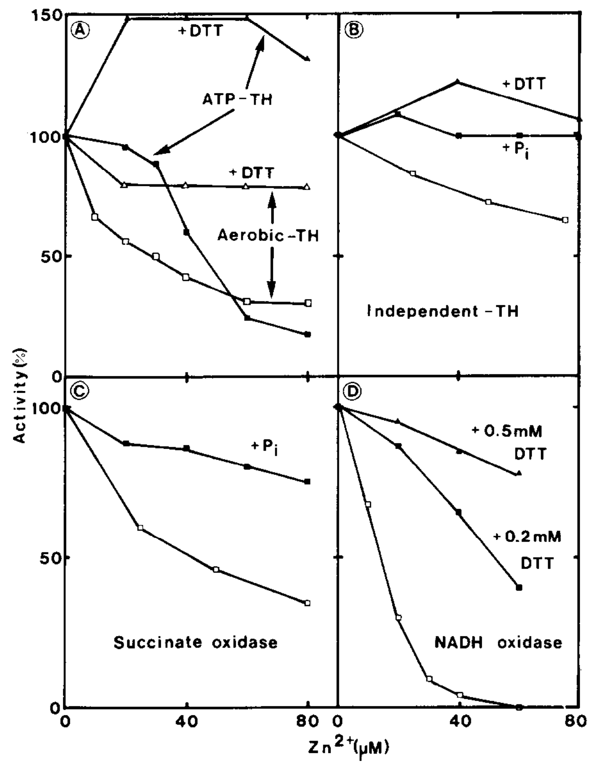

Fig. 1. Effect of Zn^{2+} on aerobic- and ATP-driven transhydrogenase (A), energy-independent transhydrogenase (B), succinate oxidase (C) and NADH oxidase (D) activities of membrane particles of *S. typhimurium.* Membrane particles (A, 1.6 mg; B, 0.2 mg; C, 0.1 mg; D, 0.4 mg protein) were preincubated at 37°C for 5 min with the indicated concentration of Zn^{2+} before the reaction was started. In experiments with dithiothreitol (DTT) this compound was added 1 min prior to the addition of Zn^{2+}. The concentrations of DDT in A and B was 0.5 mM, and that of phosphate buffer, pH 7.5, (P_i) in B and C was 50 mM. The specific activities of aerobic-driven, ATP-driven, and energy-independent transhydrogenases, succinate, and NADH oxidases were 7.2, 7.2, 233, 100 and 232 nmoles/min/mg protein, respectively.

prevent the formation of the energized state. Succinate oxidase also was inhibited by Zn^{2+} (fig. 1C) but this reaction was less sensitive than NADH oxidase. Although the results are not shown here, 0.2 mM Zn^{2+} inhibited D-lactate and succinate dehydrogenase activities by 29% and 60%, respectively. The lack of a suitable assay method prevented measurement of the effect of the inhibitor on NADH dehydrogenase. These results are consistent with the site of action of Zn^{2+} between substrate and cytochrome b_1 proposed for the respiratory chain of the related organism, *E. Coli* [13].

The sensitivity of the ATP-driven transhydrogenase, and the relative resistance of the energy-independent reaction to inhibition by Zn^{2+}, (fig. 1A,B) suggests that these metal ions must be acting either directly on the energized state or interfering with its formation from ATP. Since Zn^{2+}, up to a concentration of 1 mM, had no effect on the Ca^{2+}, Mg^{2+}-activated ATPase, the enzyme involved in the ATP-driven transhydrogenase [14], it seems likely that Zn^{2+} must be acting at the level of the energized state. In mitochondria, sulfhydryl groups have been implicated at this level [15]. The ability of dithiothreitol to reverse the inhibitory effects of Zn^{2+} on the transhydrogenase and NADH oxidase activities supports the hypothesis that Zn^{2+} acts on essential sulfhydryl groups.

Phosphate was able to prevent the inhibitory effect of Zn^{2+} on energy-independent transhydrogenase and succinate oxidase activities (fig. 1B,C). Zn^{2+} can form complexes with phosphate, AMP, ADP and ATP [16]. It seems likely that the relative resistance of the ATP-driven transhydrogenase reaction to low concentrations of Zn^{2+} when compared to the aerobic-driven reaction might be due to the removal of these metal ions as a complex with ATP.

The above results indicate that Zn^{2+} has effects on both the respiratory chain and the energized state. Thus, the observed inhibition of amino acid and sugar transport by these ions [1,2] is not necessarily due to inhibition at the level of the transport components themselves.

Acknowledgments

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