Role of Zinc in Plasma Membrane Function

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ABSTRACT The concentration of plasma zinc is the generally accepted index of zinc status. Although low plasma zinc is an essential criterion of deficiency, alone it is inadequate. To supplement this index, we sought to determine the first limiting biochemical defect in animals fed zinc-deficient diets and concluded that the limiting function is associated with a posttranslational change in plasma membrane proteins. Among the signs of zinc deficiency in rats is a bleeding tendency associated with failure of platelet aggregation, a phenomenon that correlates with impaired uptake of Ca\(^{2+}\) when stimulated. Zinc-deficient guinea pigs exhibit signs of peripheral neuropathy, and their brain synaptic vesicles exhibit impaired Ca\(^{2+}\) uptake when they are stimulated with glutamate. Red cells from zinc-deficient rats show increased osmotic fragility associated with decreased plasma membrane sulfhydryl concentration. Both phenomena are readily reversed (2 d) by dietary zinc repletion. Volume recovery is dependent on Ca-dependent K channels and the sulfhydryl redox state. Both the impaired aggregation and calcium uptake of zinc-deficient platelets are corrected by in vitro incubation of blood with glutathione. Considering the fact that plasma membranes from several cell types show impaired function that is associated with a decreased rate of calcium uptake, it is postulated that a defect in calcium channels is the first limiting biochemical defect in zinc deficiency. The calcium uptake defect and consequent impaired second-messenger function likely results from an abnormal sulfhydryl redox state in the membrane channel protein. J. Nutr. 130: 1432S—1436S, 2000.

KEY WORDS: zinc status • biochemical defect • sulfhydryl redox state • calcium channels • osmotic fragility • rats

After years of searching for an adequate index of zinc status in adult humans, investigators have not found a more efficacious measure than plasma zinc concentration. Although plasma zinc is a valuable and essential criterion of zinc status, it is by itself inadequate, because there are other physiological factors that affect plasma zinc concentrations. Zinc plays many significant roles in metabolism and is a component of numerous metalloenzymes and transcription factors. As important as these zinc proteins are in metabolism, their concentrations and activities have not proved to be useful indicators of zinc status (Bettger and O'Dell 1993), and for a good reason. Because of their high affinity for zinc, metalloproteins are not fundamental even though the measurable zinc concentration in the whole red blood cell remains unchanged (Bettger and Taylor 1986, Johanning et al. 1989, Johanning and O'Dell 1990). Decreased zinc concentration in the red cell membrane is associated with increased osmotic fragility of erythrocytes in rats (O'Dell et al. 1987, Roth and Kirchgessner 1991) and pigs (Johanning et al. 1990a). The increased fragility is readily reversed in vivo by dietary repletion but not by in vitro zinc treatment (O'Dell et al. 1987). Erythrocytes from zinc-deficient rats also have an increased sensitivity to hemolysis induced by sodium dodecyl sulfate and melittin (Patterson and Bettger 1985). Erythrocyte fragility has been studied as an index of zinc status in humans (Woodhouse et al. 1996, 1998). In experimental subjects fed a purified, low zinc diet, there was increased osmotic fragility after depletion and return to normal on repletion. However, the response was slow. The biochemical defect involved in the response of red cells to osmotic...
TABLE 1
Pathological signs of zinc deficiency

<table>
<thead>
<tr>
<th>Bleeding Tendency</th>
<th>Neuropathy; Abnormal Stance and Gait</th>
<th>Growth Failure</th>
<th>Decreased and Cyclic Food Intake</th>
<th>Dermatitis; Hair Loss</th>
<th>Impaired Parturition; Dystocia</th>
<th>Hypotension; Hypothermia</th>
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</table>

stress is unclear, but it could involve the failure of synthesis of a critical membrane component or a posttranslational modification of a protein after normal synthesis.

Alterations in the composition of plasma membranes

Numerous studies have shown that zinc deficiency alters the composition of the plasma membrane. There are changes in lipid content (Driscoll and Bettger 1997, Johanning and O’Dell 1989) and altered protein composition of the membrane skeleton extracted in low ionic strength buffer (Avery and Bettger 1991). Enzyme activities associated with the plasma membrane are depressed as well. The catalytic activities of Ca-ATPase and 5’ nucleotidase in erythrocyte membranes from rats and pigs were decreased by zinc deficiency (Johanning et al. 1990b). The rate of zinc uptake by zinc-deficient rat erythrocytes was increased (Van Wouwe et al. 1991), but there was no effect of deficiency on the uptake of glycine by oocytes or preimplantation embryos (Peters et al. 1993). Alkaline phosphatase activity was decreased in erythrocyte membranes of young men fed diets marginally deficient in zinc (Ruz et al. 1992). The increased fragility of erythrocytes appears to result from a specific plasma membrane defect; however, the question remains: What is the defect and is it general in nature, i.e., does it affect function in other cell types?

Besides the increased red cell fragility observed in experimental animals, there are numerous other gross signs of zinc deprivation. Some of the common pathological signs of zinc deficiency observed in experimental animals are listed in Table 1. The question to be addressed here is whether there is a common denominator for the diverse pathology. Consider first the bleeding tendency in pregnant rats at parturition. Association with increased bleeding tendency in the rat, there is impairment of platelet aggregation (Gordon and O’Dell 1980, Emery et al. 1990). Calcium uptake from the extracellular medium is essential for the initiation of platelet aggregation, and that function is limited in platelets from zinc-deficient rats. When platelets are stimulated with any of several agonists, such as ADP (O’Dell and Emery 1991), fluoride (Emery and O’Dell 1993) or thrombin (Xia and O’Dell 1995), aggregation and calcium uptake are impaired. Figure 1 presents a model of the mechanism by which zinc deficiency impairs platelet function. As indicated in the model, protein kinase C (PKC)^2 is an essential enzyme for platelet aggregation. Although PKC concentration is not limiting in zinc-deficient platelets (Xia et al. 1994), it is a calcium-dependent enzyme whose activity is affected by the calcium concentration in its environment. All aggregating agents tested increased internal calcium concentration, and the uptake of external calcium was impaired in zinc deficiency.

Another dramatic sign of zinc deficiency in chicks and guinea pigs is an abnormal gait and stance caused by peripheral neuropathy (O’Dell et al. 1990, 1990a). Analogous to the impaired platelet function, brain synaptic vesicles prepared from zinc-deficient guinea pigs exhibit depressed calcium uptake compared with control animals. The calcium uptake defect was observed most dramatically when the vesicles were first depolarized with potassium and then stimulated with the neurotransmitter glutamate (Browning and O’Dell 1994, 1995). Figure 2 summarizes data showing the effect of zinc status on glutamate-stimulated calcium uptake by synaptosomes prepared from guinea pig brain cortex.

Both platelets and neurons are excitable cells that might be expected to exhibit comparable responses to depolarization and to cell agonists. Importantly, similar observations have been made with nonexcitable cells, i.e., fibroblasts grown in culture. When these cells were deprived of zinc by the use of a cell-impermeant chelator, the total zinc content of the cells was unchanged but their uptake of calcium was impaired (unpublished data).

Is there a commonality between the defect in the red cell plasma membrane and that in the other cell types studied? Impairment of calcium uptake may well be the common

FIGURE 1 Model of the mechanism by which zinc deficiency impairs platelet function. Aggregation in response to stimulation with ADP, thrombin or fluoride is depressed in zinc deficiency. This is the result of failure to take up extracellular calcium, which serves as a second messenger. The increase in cytosolic calcium stimulates the activity of PKC, an enzyme whose activity is essential for aggregation.

FIGURE 2 Calcium uptake by glutamate-stimulated brain cortical synaptosomes from zinc-deficient guinea pigs is decreased. Guinea pigs were fed a low zinc diet (−ZnAL), a zinc-adequate diet ad libitum (+ZnAL) or the adequate diet restricted to maintain body weight comparable to −ZnAL (+ZnRF). The glutamate stimulus was added to synaptic vesicles in a depolarizing medium (45 mmol/L K^+). (Data from Browning and O’Dell 1994.)

2 Abbreviations: GSH, glutathione; PKC, protein kinase C; SH, sulphydryl.
thread. The hemolysis of red cells subjected to osmotic stress is the result of water uptake and the concomitant increase in volume. Volume recovery normally occurs via a mechanism that involves stretch activation of a membrane calcium channel, leading indirectly to activation of a Ca\(^{2+}\)-dependent K\(^+\) channel and the attendant loss of potassium and water (Pierce and Politis 1990). This concept is depicted by the model presented in Figure 3. It is important to note that an increase in intracellular calcium is required for activation of the potassium channel and that calcium uptake is required for volume recovery in the red blood cell. Although the rate of calcium uptake by erythrocytes in zinc deficiency has not been studied, impairment of the process by zinc deficiency would explain failure of volume recovery and the increased loss of hemoglobin. Also worthy of note is the evidence that sulfhydryl (SH) groups are essential for K\(^+\) and Cl\(^-\) transport in sheep red cells (Lauf and Mangor-Jensen 1984).

Because of the suggested relationship of osmotic fragility and the SH redox state of plasma membrane proteins, we measured osmotic fragility (hemolysis) of red cells from zinc-deficient and control rats as well as the SH concentration of red cell membranes from the same animals. As shown in Figure 4, the two parameters were highly and inversely correlated (Xia et al. 1999). Hemolysis was increased to a significantly higher level in zinc-deprived rats than in control animals within 6 d after the consumption of a low zinc diet, and the SH concentration was decreased in the same time frame (data not shown). The changes in the degree of hemolysis and the concentration of membrane protein SH were readily reversed by dietary repletion. After the rats were fed the low zinc diet for 21 d, blood was collected; then, the rats were fed a zinc-adequate diet for 2 d, and again blood was collected. As shown in Figure 5, dietary zinc repletion restored hemolysis to the control level. SH concentration was also worthy of note that the time required for depletion, ~6 d (Xia et al. 1999), is greater than that for repletion (1 d) (O’Dell et al. 1987). The explanation for this difference is not clear but probably relates to a “store” of zinc in the plasma membrane that is slowly depleted compared with the rate of repletion of zinc in the critical channel protein. This fact contributes to the greater value of fragility as an index than of plasma zinc concentration, which in rats drops markedly within 1 day after the consumption of a low zinc diet.

If the primary defect is a change in the SH redox state, it should be reversible in vitro by a reduction process that does not directly involve zinc. To explore this possibility, we used glutathione (GSH) in the platelet model that is well established in our laboratory. First, it was shown that the SH redox state is changed in platelet membranes in the same manner as in erythrocyte plasma membranes (O’Dell et al. 1997). Blood samples were taken via the tail vein; they were then fed a low zinc diet (<1 mg/kg zinc) or a control diet (100 mg/kg zinc) for 21 d; then, hemolysis and membrane SH concentration were measured. The parameters were highly correlated ($P < 0.003$). (Data from Xia et al. 1999.)
was drawn from zinc-deficient and control rats into anticoagulant in the usual manner or into the anticoagulant providing zinc-deficient red cells is readily reversed by dietary zinc repletion. Rats were fed low zinc diets for 14 d, and blood was collected from the aorta or adequate zinc diets for 14 d, and blood was collected from the aorta. Membranes were prepared from the same blood used in Figure 5, and the designations are the same. (Data from O'Dell et al. 1999.)


