Comparative effects of magnesium and zinc deficiency on alkaline phosphatase isoenzymes*)

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Zusammenfassung

Die alkalische Phosphatase ist ein Zn-haltiges Metalloenzym, das Mg zur Aktivierung benötigt; ein Mangel an Zn oder Mg verringert die Aktivität in vielen Geweben. Bei aus allen untersuchten Rattengeweben gewonnenem Enzym lassen sich mittels Polyacrylamid-Gelelektrophorese zwei Banden nachweisen, deren Laufgeschwindigkeit für jedes Gewebe charakteristisch ist. Alimentärer Mg- oder Zn-Mangel haben unterschiedliche Einflüsse auf die Aktivität der jeweiligen Bande. Bei aus Leber gewonnenem Enzym sind die Einflüsse von Mg- bzw. Zn-Mangel identisch; dagegen je nach Metall unterschiedlich bei alkalischer Phosphatase aus Serum, Niere und Femur.

Summary

Alkaline phosphatase is a zinc metalloenzyme that requires magnesium for activity and deficiencies of either metal decrease its activity in many tissues. The enzyme from all rat tissues studied separates into two bands on polyacrylamide gel electrophoresis and their mobility is characteristic of the particular tissue. Dietary deficiencies of magnesium or zinc have differential effects on the activity of individual bands of the enzyme. The effects of both deficiencies are identical with the liver enzyme, but with enzyme in serum, kidney and femur they vary with the individual metal.

Résumé

La phosphatase alcaline est un métalloenzyme zincique qui exige du Mg pour son activité, et des déficits de l'un ou l'autre de ces métaux réduisent son activité dans de nombreux tissus. L'enzyme de tous les tissus étudiés du rat se sépare en deux bandes dans l'électrophorèse en gel de polyacrylamide et leur mobilité est caractéristique d'un tissu particulier. Des déficits de la ration en Mg ou en Zn ont des effets différentiels sur l'activité des bandes isolées de l'enzyme. Les effets des deux déficits sont identiques avec l'enzyme hépatique, mais avec l'enzyme du sérum, du rein, et du fémur ils varient avec chaque métal.

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Introduction

Alkaline phosphatase [EC 3.1.3.1] is a widely distributed zinc metalloenzyme that also requires magnesium for activity. It is generally accepted that isoenzymes of alkaline phosphatase occur in different mammalian tissues, but it now appears that different forms of the enzyme may also exist within the same tissue [7, 8, 11].

Separate studies in this laboratory have recently shown that deficiencies of either magnesium [5] or zinc [1] have differential effects on individual forms of the enzyme. The present paper compares the effects of the two deficiencies on the activity of enzymic forms in several rat tissues.

Experimental

Two experiments were performed with young male Wistar albino rats that received synthetic diets deficient in either magnesium [1 mg/kg] or zinc [2.8 mg/kg]. They were pair-fed with similar animals receiving control diet [780 mg/kg of Mg and 98 mg/kg of Zn], using automatic feeding apparatus to prevent any difference in feeding pattern, which affects alkaline phosphatase activity [3]. Distilled water was provided **ad lib.** The magnesium study used rats of initial weight 100 g and lasted for 18 days, whereas the zinc study used 70 g rats and lasted for 29 days. Rats were killed by exsanguination from the heart and the metal concentrations in serum determined by atomic absorption flamephotometry.

Alkaline phosphatase was extracted by homogenizing soft tissues with Tris/HCl buffer, pH 7.4, and butan-1-ol [4], and by allowing powdered bone to autolyze in distilled water saturated with chloroform for 3 days at room temperature [2]. The activity of extracts was determined by hydrolysis of disodium phenyl phosphate at pH 10.0 [10] in medium containing 20 mg Mg/L to provide uniform conditions for activation of the enzyme.

Serum and tissue extracts were subjected to polyacrylamide gel electrophoresis on a 4 % spacer gel followed by an 8 % main gel under standard conditions as described previously [1]. Enzyme bands were detected by incubation with α-naphthyl phosphate, stained with Fast Blue BB, and measured quantitatively with a recording

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densitometer. The statistical significance of differences was assessed by Students t test.

Results

Severe growth retardation was observed in both groups of deficient animals, but particularly in the zinc-deficient rats. The serum magnesium concentration fell from 21.9 ± 0.5 to 6.5 ± 0.2 mg/L and the serum zinc concentration from 2.09 ± 0.05 to 1.27 ± 0.06 mg/L, confirming the severity of the deficiencies.

The alkaline phosphatase activity was similar in corresponding tissues from both groups of control rats, but varied between tissues. Magnesium deficiency and zinc deficiency both lowered the total alkaline phosphatase activity in serum, kidney, small intestine and femur, but had no significant effect on the activity in liver [Table 1]. The magnitude of the lowering was similar for both deficiencies in serum, but zinc deficiency tended to have a greater effect than magnesium deficiency on the other three tissues.

Tab. 1: Effects of Magnesium and Zinc Deficiency on Total Alkaline Phosphatase Activity.

Tissue	Activity as % of control animals	
	Mg-deficient	Zn-deficient
Serum	48.2*	47.1*
Liver	109.5	95.1
Kidney	76.3*	51.7*
Small intestine	78.6*	61.2*
Femur	77.1*	56.0*

* Value significantly different from control $P < 0.01$.

Electrophoresis separated the alkaline phosphatase activity from all tissues into two bands [Fig. 1]. The mobility of the individual bands was very reproducible and suggests the existence of seven different forms of the enzyme in the tissues studied. The distribution of activity between the bands varied in different tissues from control rats [Table 2]. Band one, which had the lower electrophoretic mobility, was the major component in liver and femur, but the more mobile band two was quantitatively greater in serum and kidney, and they were about equal in small intestine.

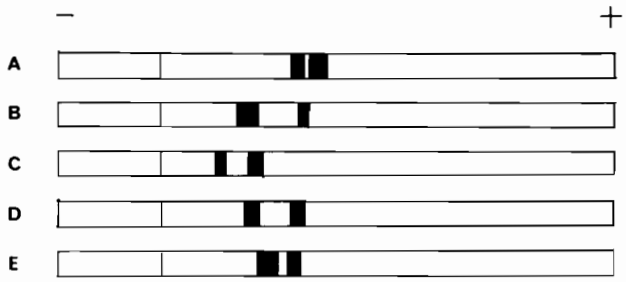

Fig. 1: Alkaline phosphatase from tissues of control rats after polyacrylamide gel electrophoresis.
A, Serum; B, liver; C, kidney; D, small intestine; E, femur.

Tab. 2: Distribution of Alkaline Phosphatase Activity among Enzyme Bands in Tissues from Control Rats.

Tissue	Activity as % of total	
	Band 1	Band 2
Serum	42	58
Liver	69	31
Kidney	40	60
Small intestine	51	49
Femur	71	29

Deficiencies of magnesium and zinc both had differential effects on the activity of particular enzyme bands [Table 3]. Magnesium deficiency significantly reduced the activity of the first enzyme bands in small intestine and femur, and the second bands in serum, liver and kidney, but it increased the activity of band one in liver and kidney, and band two in femur. Zinc deficiency, however, reduced the activity of both bands in serum, kidney and femur, although the magnitude of the inhibition varied, and it completely removed the first band found in serum from con-

Tab. 3: Effects of Magnesium and Zinc Deficiency on Activity of Individual Alkaline Phosphatase Bands.

Tissue	Band	Activity as % of control animals	
		Mg-deficient	Zn-deficient
Serum	1	84.2	0.0*
	2	24.6*	83.7*
Liver	1	131.5*	108.2*
	2	64.6*	75.8*
Kidney	1	129.3*	20.6*
	2	63.8*	87.9*
Small intestine	1	44.1*	96.3
	2	110.5	18.2*
Femur	1	33.0*	35.3*
	2	200.0*	88.1*

* Value significantly different from control $P < 0.01$.

trol rats. It selectively inhibited the second band from liver and small intestine but increased the activity of the first liver band.

Discussion

The effects of deficiencies in magnesium or zinc on alkaline phosphatase activity are superficially very similar, with the total activity of the enzyme in serum and the cellular tissues examined always being influenced in the same way by both conditions. More detailed examination of the effects on the two electrophoretically separable components of the enzyme in each tissue, however, reveals marked differences between them.

Within any one tissue the reduction in total alkaline phosphatase activity produced by either deficiency was largely due to a major action on one component of the enzyme. The major inhibitions produced by magnesium deficiency were on the second bands of serum and kidney, and the first band of small intestine, whereas the major inhibitory effects of zinc deficiency were on the first bands of serum and kidney, and the second band of small intestine. Only in femur was the major inhibitory action of both deficiencies exerted on the first enzyme band, but they differed in that magnesium deficiency increased the activity of the second band whereas zinc deficiency slightly decreased it. These differences therefore appear to be the result of selective actions of the specific metal deficiences on particular components of the enzyme.

Liver is intriguing because that was the only tissue examined where the two deficiencies produced identical effects, increasing the activity of band one and reducing the activity of band two by a corresponding amount, so that the total alkaline phosphatase activity was not significantly altered. These changes may therefore not be primary results of the mineral deficiencies but could be caused by the reduced growth rate of the deficient animals or some other secondary effect of nutritional failure.

The difference between the various bands with alkaline phosphatase activity is unknown and they do not appear to correlate with the results of any previous work. Their consistent electrophoretic mobilities suggest the existence of seven different forms of the enzyme that associate differently in individual tissues and may be characteristic of the particular tissue. Preliminary attempts in this laboratory to separate the two components from liver on a larger scale by gel filtration indicate that they have a molecular weight of 150,000 or greater. This is consistent with the accepted value of about 150,000 for the molecular weight of highly purified alkaline phosphatase from rat and human liver [6, 9], as well as other tissues, and suggests that the bands must represent different forms of the holoenzyme rather than subunits.

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