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EVIDENCE FOR THE PHYSIOLOGICAL FUNCTION OF ALKALINE PHOSPHATASE

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The evidence for a possible role of intestinal alkaline phosphatase in the physiologic state is offered in this paper. A phosphate transport system operating at a physiological pH is found in the upper part of the small intestine where the alkaline phosphatase is maximally concentrated. The phosphate transport was affected by various inhibitors and antiserum to rat intestinal alkaline phosphatase. In conclusion, alkaline phosphatase may function not only as a hydrolytic enzyme of phosphoester but also as a phosphate transporter in the physiologic state.

KEYWORDS——alkaline phosphatase; rat intestine; physiological function; hydrolytic enzyme; phosphate transporter

Alkaline phosphatase is known to catalyze the hydrolysis of various phosphate compounds and analyses of this enzyme are performed under optimal pH conditions using synthetic phosphomonoesters as substrates. Alkaline phosphatase is found in abundance at the absorption site of various materials.[1-3] As functions of alkaline phosphatase, several investigators have suggested hydrolysis of phosphoesters,[4] phosphate transferase activity,[5,6] transport of phosphate[7,8] and so on. Although alkaline phosphatase was discovered more than 80 years ago, there is no positive evidence for its physiological function. In this paper, we now present evidence for a possible role of intestinal alkaline phosphatase as a phosphate transporter in the physiologic state.

Utilizing rat intestine, the relation between intestinal alkaline phosphatase activity and phosphate transport was investigated. As shown in Fig. 1, the amount of phosphate transported into the serosal lumen of everted rat intestine was maximal at the upper part of the jejunum. The sites of intestinal absorption of most materials are not all at the upper part of the jejunum. For example, lipids, bile acids, vitamin B_{12} and gluculonated drugs are absorbed at the ileum.[9] Therefore, these findings indicate that phosphate is preferentially transported at the upper part of the jejunum. The correlation between the enzyme activity and the amount of transported phosphate was excellent ($r=0.990$). Moreover, in a previous paper, we reported finding by radioimmunoassay that the activity of human placental

alkaline phosphatase was in parallel with the protein content.[10] From these findings it appeared that there was a phosphate transport system in the upper part of the jejunum and alkaline phosphatase was localized at the very site of phosphate transport. The effect of the pH changing from 6.5 to 8.0 in the mucosal fluid on the phosphate transport was also investigated. As shown in Table I, the amount of phosphate transported into the serosal lumen of everted rat intestine was maximal at pH 7.4 which is physiological.

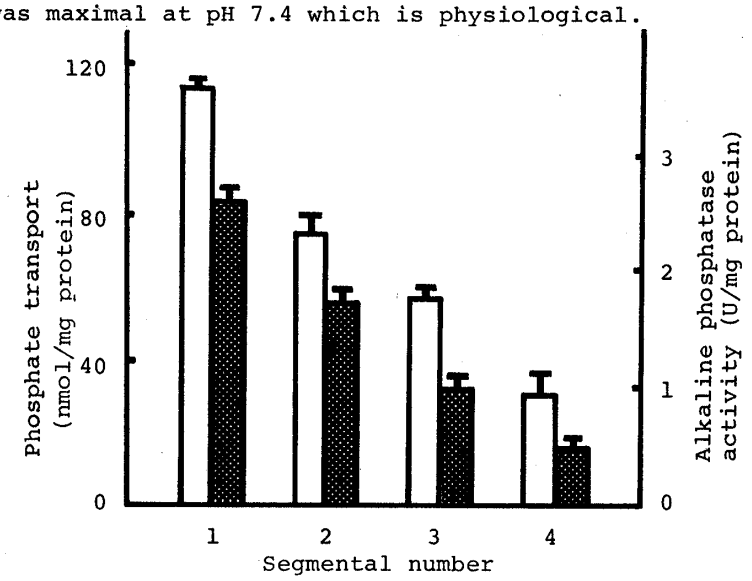

Fig. 1. Relation between Rat Intestinal Alkaline Phosphatase Activity and Phosphate Transport

Intestinal segments (about 7 cm) of the lower duodenum were quickly removed from male Wistar rats (weighing 180 to 200 g), washed gently with 20 mM Tris-HEPES buffer (pH 7.4) containing 0.1 M NaCl and 0.1 M mannitol, and everted by means of a glass tube. A glass tube was inserted in the end of the everted intestine and tied securely with thread. The glass tube was connected with a Tygon tube. The intestinal lumina were immersed in 1 mM disodium hydrogen phosphate in the above buffer maintained at 37°C and saturated with 95% O_2 and 5% CO_2. Six ml of the above buffer was circulated through the serosal lumen of everted intestine with a roller pump at 0.5 ml/min. The phosphate content in the circulating buffer was determined by the method of Fiske and SubbaRow.[11] Alkaline phosphatase activity was determined by the method of Bessey and Lowry[12] using p-nitrophenyl-phosphate as a substrate. Protein was measured by the method of Lowry et al.[13] with bovine serum albumin as a standard. The values are represented as the amount of transported phosphate after 90 min of incubation. The distance from the duodenum to the ileum is denoted by 1-4. The amount of transported phosphate (□) and alkaline phosphatase activity (▩) are shown. The mean values ± S.D. were derived from three experiments.

Table I. Effect of pH on the Phosphate Transport into Everted Rat Intestine

pH	Phosphate transport (nmol/mg protein)
6.5	57.4 ± 3.9
7.0	59.0 ± 3.2
7.4	84.5 ± 7.4
8.0	54.8 ± 2.9

The incubation medium consisted of 20 mM Tris-HEPES, 0.1 M NaCl, 0.1 M mannitol and 1 mM disodium hydrogen phosphate at the indicated pH. Other conditions were as described in the legend for Fig. 1. The values represent the amount of phosphate transported after 60 min of incubation. Mean values ± S.D. derived from three experiments are given.

Also, the amount of phosphate transported through everted rat intestines showed a

saturation curve which followed Michaelis-Menten kinetics, and an apparent K value for phosphate was calculated to be 1.0×10^{-4} M. This value was in good agreement with the Ki (1.5×10^{-4} M) calculated from the inhibition of the activity of rat intestinal alkaline phosphatase by phosphate at pH 7.4. When disodium phenylphosphate instead of inorganic phosphate was dissolved in the mucosal fluid as a substrate, the phosphate liberated from the substrate was transported into the serosal lumen of everted rat intestine as shown in Fig. 2. The amount of phosphate transported into the serosal lumen of everted rat intestine in the presence of various inhibitors or anti-rat intestinal alkaline phosphatase antiserum was also examined. The inhibitors presented in Fig. 2 significantly suppressed the phosphate transport. Especially the antiserum to rat intestinal alkaline phosphatase suppressed the phosphate transport. But normal rabbit serum did not suppress the phosphate transport. The binding of antibody to the enzyme has no influence on the enzyme activity.[14,15] From these results, it seems that rat intestinal alkaline phosphatase has an ability to pick up phosphate as a phosphate transporter in the physiologic state.

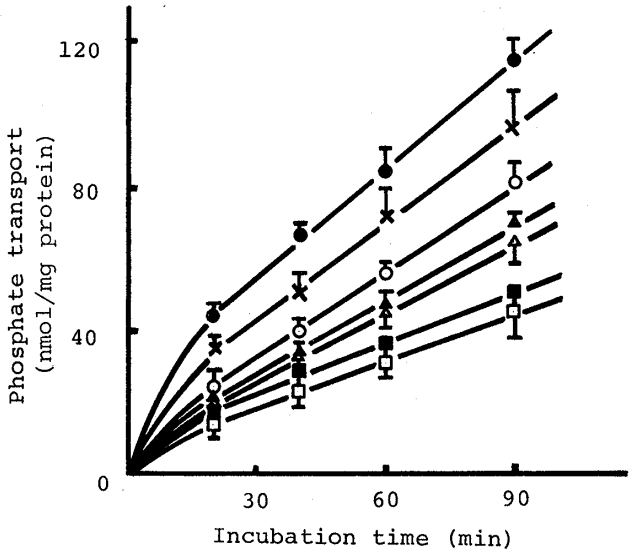

Fig. 2. Effect of Various Inhibitors on the Phosphate Transport into Everted Rat Intestine

The incubation medium consisted of 20 mM Tris-HEPES (pH 7.4), 0.1 M NaCl, 0.1 M mannitol, 1 mM disodium hydrogen phosphate and various inhibitors indicated. ● , control; ○ , 1 mM vanadate; ▲ , 1 mM KCN; △ , 10 mM L-phenylalanine; ■ , 1 mM arsenate; □ , antiserum to rat intestinal alkaline phosphatase; × , 4.2 mM disodium phenylphosphate instead of 1 mM disodium hydrogen phosphate in the control. Other conditions were as described in the legend for Fig. 1. Mean values ± S.D. derived from three experiments are given. Antiserum to rat intestinal alkaline phosphatase was obtained from rabbits immunized with the purified rat intestinal alkaline phosphatase in the same manner of the previous paper.[16]

Furthermore, the Ki values of competitive inhibitors, phosphate, arsenate (4.8×10^{-6} M) and vanadate (3.3×10^{-6} M), at pH 7.4 were lower by two orders of magnitude than those at the optimal pH, 9.5. In particular, the binding of phosphate to the alkaline phosphatase molecule was thought to be much tighter than that at the optimal pH and this fact may be advantageous for picking up phosphate. On the other hand, the Ki values of non-competitive inhibitor, L-phenylalanine (4.6×10^{-3} M) and chelating reagent, potassium cyanade (3.3×10^{-3} M), at pH 7.4 were not different

from those at the optimal pH. These reagents also suppressed the phosphate transport. L-Homoarginine and ouabain which are the inhibitors of liver alkaline phosphatase and sodium- and potassium-dependent ATPase, respectively, showed no effects on the phosphate transport under these conditions.
However, the suppression ability of the phosphate transport by competitive or other inhibitors was unaccountable from the kinetic parameters, the Ki values of these inhibitors. These discrepancy may be explained on the basis that the kinetic parameters of the hydrolytic activity of alkaline phosphatase can not be applied to that of the phosphate transport. Indeed, the velocity of the phosphate transport (1.45×10^{-9} mol·mg^{-1}·min^{-1}) was 1 : 23000 compared with that (3.3×10^{-6} mol·mg^{-1}·min^{-1}) of the hydrolytic activity of rat intestinal alkaline phosphatase towards p-nitrophenylphosphate. Also, the phosphate transport was not suppressed by these inhibitors at the given concentrations of Ki values.

In these findings of the present study, especially, the antiserum to rat intestinal alkaline phosphatase suppressed the phosphate transport. This fact may be attributed to the inhibition of conformational change induced by the binding of phosphate.[17] Taking all things into consideration, it is suggested that alkaline phosphatase may function not only as a hydrolytic enzyme of phosphomonoesters but also as a phosphate transporter in the physiologic state. Moreover, the direction of phosphate transport is probably controlled by some conditions, mainly by pH.

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