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# Alkaline phosphatase activity does not mediate phosphate transport in the renal-cortical brush-border membrane

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We studied (1) the effect of primary modulators of phosphate transport, namely the hypophosphataemic mouse mutant (*Hyp*) and low-phosphorus diet, on alkaline phosphatase activity in mouse renal-cortex brush-border membrane vesicles and (2) the effect of several primary inhibitors of alkaline phosphatase on phosphate transport. Brush-border membrane vesicles from *Hyp*-mouse kidney had 50% loss of $Na^+$-dependent phosphate transport, but only 18% decrease in alkaline phosphatase activity. The low-phosphorus diet effectively stimulated $Na^+$/phosphate co-transport in brush-border membrane vesicles (+ 118%), but increased alkaline phosphatase activity only slightly (+ 13%). Levamisole (0.1 mM) and EDTA (1.0 mM) inhibited brush-border membrane-vesicle alkaline phosphatase activity by 82% and 93% respectively, but had no significant effect on $Na^+$/phosphate co-transport. We conclude that alkaline phosphatase does not play a direct role in phosphate transport across the brush-border membrane of mouse kidney.

Alkaline phosphatase is a ubiquitous enzyme of unknown function (McComb *et al.*, 1979). A role for the enzyme in the transmembrane transport of phosphate ($P_i$) has been proposed by various investigators. We examined this relationship in mouse renal brush-border membranes in two ways: first, with modulators of $P_i$ transport, namely the hypophosphataemic mouse mutant (*Hyp*) (Eicher *et al.*, 1976), in which there is a selective deficiency of $Na^+/P_i$ co-transport in the renal brush-border membrane (Tenenhouse *et al.*, 1978), and environmental deprivation of phosphorus, in which phosphate transport is stimulated (Tenenhouse & Scriver, 1979*a*); second, with inhibitors of alkaline phosphatase activity, namely levamisole (Van Belle, 1972), EDTA (George & Kenny, 1973), L-phenylalanine (George & Kenny, 1973) and disodium 1-hydroxyethane-1,1-diphosphonate. We found that alkaline phosphatase is not an important mediator of $P_i$ transport in the renal brush-border membrane.

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## Experimental

### *Materials*

Carrier-free $^{32}P$ (catalogue no. NEX-054), D-[$^3$H]glucose (catalogue no. NET-050) and Aquasol-2 were purchased from New England Nuclear, Boston, MA, U.S.A.; Millipore filters, type HAWP 02500 (0.45 $\mu$m) were from Millipore Corp., Bedford, MA, U.S.A. Levamisole hydrochloride was from Aldrich Chemical Co., Milwaukee, WI, U.S.A. *p*-Nitrophenyl phosphate (disodium salt) and *p*-nitrophenol stock solution were from Sigma, St. Louis, MO, U.S.A. Diethanolamine and all common chemicals were purchased from Fisher Scientific. Disodium 1-hydroxyethane-1,1-diphosphonate was a gift from Dr. G. S. Hassing of Procter and Gamble Co., Cincinnati, OH, U.S.A.

### *Animals*

The initial breeding pairs of *Hyp* mice were generously provided by Dr. Eva Eicher, Jackson Laboratory, Bar Harbor, ME, U.S.A. Animals were subsequently bred in our own facilities. Male Swiss white mice, used in the studies with EDTA, phenylalanine, hydroxyethanediphosphonate and



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levamisole, were purchased from Canadian Breeding Farms, Montreal, Que., Canada.

*Diets*

Mice between 24 and 50 weeks of age were grouped and fed on diets of defined composition for 2 weeks before study, as reported elsewhere (Tenenhouse & Scriver, 1979*a*). Diets were obtained from ICN, Cleveland, OH, U.S.A.; the control diet (0.4% phosphorus, w/w) was prepared at ICN by adding sodium phosphate to the ICN low-phosphorus diet (0.03%).

*Transport studies*

Brush-border membrane vesicles were isolated from renal cortex by the method of Booth & Kenny (1974) with slight modifications, and their purification was evaluated as described elsewhere (Tenenhouse *et al.*, 1978; Tenenhouse & Scriver, 1978). Net uptake of labelled $P_i$ by freshly prepared brush-border membrane vesicles was measured by the Millipore-filter technique (Tenenhouse *et al.*, 1978; Tenenhouse & Scriver, 1978). Simultaneous transport of D-glucose served as an internal control. The effects of EDTA and levamisole on $P_i$ transport and on alkaline phosphatase activity were studied in pretreated brush-border membrane vesicles washed twice with 40ml of 300mM-mannitol/20mM Tris/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], pH7.4, containing either EDTA (1mM) or levamisole (0.1mM). Membranes were then suspended in 2ml of the same buffer at a final brush-border membrane-vesicle protein concentration of 3–6mg/ml. Levamisole or EDTA was present also in the incubation buffer.

The effect of any single variable was always compared in control and experimental brush-border membrane vesicles prepared on the same day. The data in Table 1 represent $Na^+$-dependent uptake exclusively, i.e. uptake in the presence of a KCl gradient was subtracted from uptake in a NaCl gradient. The concentrations of $P_i$ and glucose were 100$\mu$M and 10$\mu$M respectively in incubations with brush-border membrane vesicles prepared from *Hyp* mice and mice on low-phosphorus diets. In experiments with EDTA and levamisole, the concentrations of $P_i$ and glucose were 15$\mu$M and 10$\mu$M respectively.

*Alkaline phosphatase assay*

Alkaline phosphatase activity of brush-border membrane vesicles was measured with 0.001M-*p*-nitrophenyl phosphate in 1.0M-Tris/HCl, pH8, or with 0.005M-*p*-nitrophenyl phosphate in 1.0M-diethanolamine, pH10. Assays were performed at 20°C in 1ml of reaction mixture, to which 10$\mu$l of membrane solution was added. The increase in $A_{410}$ was recorded; a cuvette containing *p*-nitrophenyl phosphate in the appropriate buffer without brush-border membrane vesicles served as a blank. The molar absorption coefficient for *p*-nitrophenol was $1.79 \times 10^4$ in 1.0M-Tris/HCl, pH8, and $1.9 \times 10^4$ in 1M-diethanolamine, pH10. One unit of alkaline phosphatase is equivalent to 1nmol of *p*-nitrophenol cleaved/min per mg of protein.

## Results

*Effect of Hyp mutation*

Renal-cortical brush-border membrane vesicles prepared from *Hyp* mice have only half the normal $Na^+$-dependent $P_i$-transport activity ($P < 0.001$) (Table 1). The deficiency of transport was specific for $P_i$, and simultaneous uptake of D-glucose by brush-border membrane vesicles was similar in mutant and normal mice. By comparison, alkaline phosphatase activity was only slightly decreased in the mutant membranes (Table 1); the decrease (18%, $P < 0.02$) was much less than the corresponding decrease in $P_i$ transport (53%, $P < 0.001$).

*Effect of phosphorus deprivation*

Renal brush-border membrane vesicles prepared from mice exposed to phosphorus deprivation had increased ($P < 0.001$) $Na^+$-dependent transport of $P_i$ (Table 1); D-glucose transport was not enhanced. By comparison, only a slight increase (+13%, $P < 0.05$) in alkaline phosphatase activity of brush-border membrane vesicles was observed in the phosphorus-deprived animals (Table 1).

*Effect of alkaline phosphatase inhibitors*

When brush-border membrane vesicles were washed and suspended in 1mM-EDTA, the agent had no effect on $Na^+$-dependent $P_i$ transport or $Na^+$-dependent glucose transport (Table 1). On the other hand, alkaline phosphatase activity was strongly inhibited. Inhibition of alkaline phosphatase activity occurred with or without EDTA pretreatment of brush-border membrane vesicles. Similar results were obtained with levamisole, which did not inhibit $Na^+$-dependent $P_i$ transport significantly, whereas it was a potent inhibitor of alkaline phosphatase activity (Table 1).

L-Phenylalanine and hydroxyethanediphosphonate both proved not to be useful probes, but for different reasons. L-Phenylalanine (at 10mM) inhibited alkaline phosphatase activity of brush-border membrane vesicles by 30%, as reported previously (George & Kenny, 1973); it also inhibited the $Na^+$-dependent transport of $P_i$ by 59% and of D-glucose by 61%. Because brush-border membrane-vesicle transport of phenylalanine requires a $Na^+$ gradient (Evers *et al.*, 1976), it is likely that the effect of the amino acid on other $Na^+$-dependent

Table 1. *Effect of mutation, diet and alkaline phosphatase inhibitors on mouse renal brush-border membrane-vesicle phosphate and glucose transport and alkaline phosphatase activity*

Details of experiments are described in the Experimental section. Transport data represent the means ± S.E.M. of six determinations. Alkaline phosphatase data represent the means ± S.E.M. of three determinations on at least two brush-border membrane-vesicle preparations. Significance of difference between experimental and control data was calculated by Student's *t* test.

| | | Transport | | Alkaline phosphatase | |
|---|---|---|---|---|---|
| Experimental variable | Time | Phosphate (pmol/mg of protein) | Glucose (pmol/mg of protein) | pH for assay | Activity (units) |
| Genetic: Control | 30s | 663 ± 51 | 164 ± 19 | 8 | 142 ± 8 |
| *Hyp* | 30s | 314 ± 9 ($P < 0.001$) | 163 ± 11 | 8 | 116 ± 5 ($P < 0.02$) |
| Diet: Control | 30s | 863 ± 44 | 187 ± 23 | 10 | 8100 ± 200 |
| Low-P | 30s | 1878 ± 139 ($P < 0.001$) | 135 ± 19 ($P < 0.05$) | 10 | 9100 ± 200 ($P < 0.05$) |
| Inhibitors: Control | 15s | 244 ± 6 | 77 ± 3 | 10 | 11200 ± 200 |
| EDTA (1 mM) | 15s | 247 ± 9 | 67 ± 2 | 10 | 790 ± 50 ($P < 0.001$) |
| Levamisole (0.1 mM) | 15s | 230 ± 8 | 63 ± 3 | 10 | 2050 ± 140 ($P < 0.001$) |

transport processes reflects competition for the common driving force.

Hydroxyethanediphosphonate, an analogue of pyrophosphate, inhibits bone resorption and the activity of several phosphohydrolases (Felix *et al.*, 1976). The substance, at 1 mM, inhibited alkaline phosphatase activity (at pH 10) in brush-border membrane vesicles by 48%; it also inhibited $Na^+$-dependent $P_i$ transport. The appropriate double-reciprocal plot (not shown) indicated that the diphosphonate is a competitive inhibitor of $Na^+$-dependent $P_i$ transport. The apparent $K_i$ for the diphosphonate, under our experimental conditions, was $1.8 \times 10^{-4}$ M; the apparent $K_m$ for $P_i$ was $0.65 \times 10^{-4}$ M, in agreement with our previous work (Tenenhouse & Scriver, 1979*a*).

## Discussion

The *Hyp* mutation is associated with impaired phosphate reabsorption by kidney *in vivo* and partial deficiency of $Na^+$-dependent $P_i$ transport in brush-border membrane vesicles (Tenenhouse *et al.*, 1978; Tenenhouse & Scriver, 1978). Alkaline phosphatase activity is not diminished to the same extent in *Hyp* brush-border membrane vesicles; expression of the *Hyp* allele is largely confined to $P_i$ transport rather than to alkaline phosphatase activity. Dietary deprivation of phosphorus is a potent signal for enhancement of $P_i$ transport in the adapted kidney, both *in vivo* and *in vitro* (Tenenhouse & Scriver, 1979*a*,*b*). There is little adaptation in alkaline phosphatase activity under these conditions. Our findings, in the mouse, agree well with those reported by Stoll *et al.* (1979) for the rat. However, Kempson & Dousa (1979) observed that phosphorus deprivation in the rat stimulated alkaline phosphatase activity by 33% and $P_i$ transport by 90%, and they suggested that the two findings were related. Their data differ from ours in the greater stimulation of alkaline phosphatase activity and lower stimulation of $P_i$ transport.

The effect of levamisole *in vitro* is not in accord with its effect *in vivo* on phosphate reabsorption (Plante *et al.*, 1977). Intra-arterial infusion of levamisole in the anaesthetized dog is said to increase phosphaturia and to inhibit renal alkaline phosphatase. The discrepancy between the effects *in vitro* and *in vivo* may lie in the range of metabolic effects that must arise when levamisole is used *in vivo* and which may alter renal reabsorption of $P_i$ secondarily.

Alkaline phosphatase is a microvillar enzyme (Kenny & Booth, 1978). Under conditions where enzymes associated with brush-border membranes were cleaved by papain treatment, there was no release of alkaline phosphatase (George & Kenny, 1973). Noronha-Blob (1979) showed that papain treatment of rabbit renal brush-border membranes decreased the initial rate of the $Na^+$-dependent $P_i$ transport by 60% without influence on the transport of D-glucose or L-proline and without significant alteration of alkaline phosphatase activity.

Whereas each of the aforementioned findings suggests that alkaline phosphatase activity in the renal brush-border membrane is not directly involved in phosphate transport by renal epithelium, our studies with hydroxyethanediphosphonate might

indicate otherwise. However, inhibition of alkaline phosphatase and $P_i$ transport in the brush-border membrane by the diphosphonate need not imply that both activities are attributable to a single protein.

One further matter deserves consideration. Since isoenzymes of alkaline phosphatase exist, and intestine and kidney have different forms of the enzyme (Seargeant & Stinson, 1979), it is possible that the relationship between $P_i$ transport and alkaline phosphatase activity in the intestine (Moog & Glazier, 1972; Shirazi *et al.*, 1978) differs from that described here for kidney.

**Note Added in Proof (Received 16 June 1980)**

Storelli & Murer (1980) found no correlation between alkaline phosphatase activity and transport of phosphate in isolated rat renal brush-border membrane vesicles. These findings support our conclusions.

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## References

Booth, A. G. & Kenny, A. J. (1974) *Biochem. J.* **142**, 575–581

Eicher, E. M., Southard, J. L., Scriver, C. R. & Glorieux, F. H. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4667–4671

Evers, J., Murer, H. & Kinne, R. (1976) *Biochim. Biophys. Acta* **426**, 598–615

Felix, R., Russell, R. G. G. & Fleisch, H. (1976) *Biochim. Biophys. Acta* **429**, 429–438

George, S. G. & Kenny, A. J. (1973) *Biochem. J.* **134**, 43–57

Kempson, S. A. & Dousa, T. P. (1979) *Life Sci.* **24**, 881–888

Kenny, A. J. & Booth, A. G. (1978) *Essays Biochem.* **14**, 1–44

McComb, R. B., Bowers, G. N. & Posen, S. (1979) *Alkaline Phosphatase*, Plenum Publishing Corp., New York

Moog, F. & Glazier, H. S. (1972) *Comp. Biochem. Physiol.* **42A**, 321–336

Noronha-Blob, L. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 3204

Plante, G., Lehoux, J. & Petitclerc, C. (1977) *Clin. Res.* **25**, 444A

Seargeant, L. E. & Stinson, R. A. (1979) *Nature (London)* **281**, 152–154

Shirazi, S. P., Colston, K. W. & Butterworth, P. J. (1978) *Biochem. Soc. Trans.* **6**, 933–935

Stoll, R., Kinne, R. & Murer, H. (1979) *Biochem. J.* **180**, 465–470

Storelli, C. & Murer, H. (1980) *Pflügers Arch.* **384**, 149–153

Tenenhouse, H. S. & Scriver, C. R. (1978) *Can. J. Biochem.* **56**, 640–646

Tenenhouse, H. S. & Scriver, C. R. (1979*a*) *Can. J. Biochem.* **57**, 938–944

Tenenhouse, H. S. & Scriver, C. R. (1979*b*) *Nature (London)* **281**, 225–227

Tenenhouse, H. S., Scriver, C. R., McInnes, R. R. & Glorieux, F. H. (1978) *Kidney Int.* **14**, 236–244

Van Belle, H. (1972) *Biochim. Biophys. Acta* **289**, 158–168