Selective Removal of Alkaline Phosphatase from Renal Brush-Border Membrane and Sodium-dependent Brush-Border Membrane Transport

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Na+-gradient-dependent transport of phosphate (Pi), glucose, and proline was studied in renal brush-border membranes (BBM) from which alkaline phosphatase was released by treatment with phosphatidylinositol-specific phospholipase C. BBM were prepared from rabbit kidney cortex in the form of large brush-border membrane sheets (BBMS). Incubation of BBMS with bacterial phosphatidylinositol-specific phospholipase C resulted in selective release (up to 90%) of the alkaline phosphatase from BBM; in contrast, activities of leucine aminopeptidase, γ-glutamyltranspeptidase, and maltase were not affected. Polytom homogenization of BBMS leads to the formation of brush-border membrane vesicles (BBMV) capable of concentrative uptake of solutes. BBMS and BBMV were prepared from kidneys of rabbits fed either a high (1.2% P), low (0.07% P), or normal P diet. Enzymatic deletion of alkaline phosphatase from BBMV prepared from animals fed the low P diet resulted in a marked increase in Na+-gradient-dependent uptake of Pi. No such effect was observed in BBMV from animals fed the normal or high P diets. These experiments indicate that the presence of alkaline phosphatase in BBM is not required for Na+-gradient-dependent transport of Pi, glucose, and proline. Likewise, the adaptive increase in BBM transport of Pi, elicited in response to low dietary P intake does not depend on the presence of, or increase in, alkaline phosphatase activity. Our findings argue against a direct involvement of alkaline phosphatase in Na+-dependent Pi transport across the renal BBM. It is not excluded, however, that alkaline phosphatase might play a role in the modulation of Pi transport.

The Na+-gradient (extravesicular > intravesicular, Na+-Pi > Na+) dependent uptake of Pi across luminal BBM1 is considered to be a major step in transport of Pi across the wall of the proximal tubule in the mammalian kidney (i). While many basic properties of this secondary active transport of Pi, and its regulation have been recently described (1-4), the molecular basis of BBM transport of Pi, remains unknown.

Much controversy has been generated recently around the question of whether alkaline phosphatase, one of the typical BBM enzymes (5), may play a role in BBM uptake of Pi, and/or its regulation (1, 6-9). The role of alkaline phosphatase in the transport of Pi, across membranes in general was first proposed based on studies of unicellular organisms (10-13) and was later extended by other studies on Pi transport across mammalian epithelia (1, 6, 7, 14-17).

In several pathophysiological states in which renal BBM transport of Pi is specifically diminished, the enzymatic activity of alkaline phosphatase, but not that of other typical BBM enzymes, is also specifically decreased (1, 6, 7, 9, 16-19). In contrast, in nutritional P deprivation, both in bacterial (10-13) and in mammals (14, 16, 17), an increased P transport was found to be associated with the increased alkaline phosphatase activity (10, 11, 14, 17). The parallel increase in BBM transport of P, and in BBM activity of alkaline phosphatase in response to low P diet was blocked by administration of actinomycin D, an inhibitor of de novo protein synthesis (16).

No consensus has been reached, however, concerning the possible role of alkaline phosphatase in the BBM transport of Pi, or its regulation (1, 6-9). Some investigators argue that positive associations between changes in alkaline phosphatase and in the BBM transport of P, may not be related to each other at all (8, 9), because the extent of changes observed are not quantitatively the same. Also, changes in BBM transport of P, reportedly preceded changes in the activity of alkaline phosphatase (20).

Attempts to elucidate the role of alkaline phosphatase with the use of specific inhibitors added in vitro have also been inconclusive (1, 6, 8, 9, 21). Some inhibitors of alkaline phosphatase had equivocal or no effect on the transport of Pi, (1, 8, 9, 21, 22), while others inhibited alkaline phosphatase and Pi transport in parallel (9, 23). Interpretation of such studies with inhibitors is limited by the fact that alkaline phosphatase activity is often measured under pH conditions which vastly differ from those prevailing in vivo (6, 8, 9) and also is assayed using artificial substrates (1, 6, 23) which may or may not resemble the yet to be identified natural substrate(s) for this enzyme. It is also conceivable that not the catalytic site of alkaline phosphatase, but some other region of the alkaline phosphatase molecule, might relate to the Pi transport.

To explore the possible role of alkaline phosphatase in the Na+-gradient-dependent BBM transport of Pi, across renal BBM in a more direct and definitive way, in the present

1 The abbreviations used are: BBM, brush-border membranes; BBMV, brush-border membrane vesicles; BBMS, brush-border membrane sheets; Pi-PLC, phosphatidylinositol-specific phospholipase C; Hepes, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid.
experiments we selectively removed membrane-bound alkaline phosphatase by enzymatic digestion with PI-PLC (24-27). This enzyme splits off myoinositol phosphate from phosphatidylinositol and specifically releases alkaline phosphatase from the membranes of various tissues (24-27).

The results of the present study show that removal of over 90% of the alkaline phosphatase from the matrix of BBM had no diminishing effect on Na+-gradient-dependent transports of P<sub>1</sub>, d-glucose, or L-proline across BBM. On the contrary, this treatment in fact increased the P<sub>1</sub> transport of BBM isolated from animals adapted to a low P diet.

METHODS

BBM were isolated from kidneys of fresh-killed New Zealand albino rabbits. Rabbit kidney was selected because it permits preparation of BBM in the form of large sheets (28) and also because membranes from this species had been used previously for digestion studies with PI-PLC (24, 26). Unless specified otherwise, rabbits were maintained on a diet containing normal levels of P (0.7% P; Purina Rabbit Chow) and ad libitum drinking of tap water. In experiments where the effects of dietary P were examined, rabbits were fed a low P diet (0.07% P; ICN Pharmaceuticals Inc., Cleveland, OH) or fed the same diet supplemented with a mixture of Na and K salts of phosphate (ratio of monobasic:dibasic salts, 1:4) to attain either a normal or low dietary phosphorus content.

Preparations of Brush-Border Membranes—BBM were prepared as large fragments of proximal luminal plasma membrane with preserved microvillar structure (28). In this preparation referred to in this text as "brush-border membrane sheets," both the cytoplasmic (intracellular) and luminal (extracellular) membrane sides were accessible to the bathing medium. BBM were also further processed (29, 30) to form small fragments of microvilli, which spontaneously sealed into vesicles (30). These preparations, denoted as "brush-border membrane vesicles," show P<sub>1</sub> transport characteristics, i.e. concentration-dependent, transient and dependent on the presence of Na<sup>+</sup> (extravesicular > intravesicular)-gradient. The time course of P<sub>1</sub> uptake (Fig. 1) is identical with that observed with BBMV prepared from kidneys of rabbit (13, 31) and other animals (2, 3, 7, 9, 22, 31) using the divalent cation precipitation method.

BBMS were prepared using a modified method of Thuneberg and Rostgaard (28) as follows (Fig. 2). The renal cortical tissue was weighed and transferred to 5 volumes of ice-cold 0.15 M NaCl-Hepes buffer (pH 7.4), and the cortex was dissected free of all noncortical tissue. As a rule, cortical tissue pooled from six kidneys was used for each preparation.

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Preparations of Brush-Border Membrane Vesicles—BBMV were prepared from BBMS as described above (Fig. 2) by a modification of the method of Booth and Kenny (29). The suspension of BBMS in 0.5 M sucrose was diluted with 5 mM Tris Hepes, pH 8.5, to a sucrose concentration of 0.3 M and then subjected to ultrasonic treatment with a more tight-fitting Potter-Elvehjem Teflon pestle. The homogenate was layered over 1.4 M sucrose (11.30 ml) in 38.5-ml centrifugation tubes, the interface being lightly stirred with the tip of a Pasteur pipette. The tubes were centrifuged for 60 min at 90,000 × g in a Beckman L-28 ultracentrifuge using an SW-27 rotor. The interfacial layer containing the BBMS and the supernatant were removed, mixed, and then centrifuged at 4,000 × g for 15 min. The pellet was resuspended in 0.5 M sucrose by gentle shaking (1 ml/g of original tissue) and centrifuged at 32,000 × g for 5 min. The BBMS accumulated as a pink, loose upper layer over a darker hard-packed pellet. After carefully removing the bulk of the supernatant, the BBMS fraction was removed from the lower dark pellet and by gentle mixing with some of the remaining supernatant and then resuspended at 4,000 × g for 10 min. The pellet as obtained was resuspended in 0.5 M sucrose (0.5 ml/g of original tissue) and further purified by successive resuspension (with 0.5 M sucrose) and sedimentation at 4,000 × g, 2,000 × g, and 1,000 × g, with centrifugation times of 10 min each (28). The BBMS fraction thus obtained was confirmed by phase contrast microscope to be fragments of BBM with a long, parallel oriented microvillar structure. Such a preparation was enriched in the BBM enzymes, but incapable of uphill uptake of 32P, (Table 1).

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to three consecutive 30-s homogenizations using a Polytron with 30-s intervals between each. After pelleting the unhomogenized fragments (debris) by centrifugation at 1,500 × g (12 min), the BBMV in the supernatant were pelleted at 45,000 × g (20 min) and resuspended in 500 mM mannitol, 5 mM Hepes, 5 mM Tris, pH 8.5. Solute transport in BBMV was measured using the rapid filtration technique (3) described in earlier studies (16-18, 31, 32, 36, 38). The concentration of solute tracers were 0.1 mM 32P, 0.05 mM D-[3H]glucose, and 0.025 mM L-[3H]proline. The BBMV fraction was further enriched in BBM enzymes, compared to BBMS (Table I), and exhibited uphill uptake of 32P (Table I; Fig. 1). Volumes and protein content of each fraction were monitored. All subsequent enzymatic digestions of BBMS with PI-PLC, and transport measurements, were conducted with fresh BBMV preparations. Aliquots from these preparations were quickly frozen in dry ice and stored at −80 °C for determination of BBM enzyme activities and protein content.

**Phosphatidylinositol-specific Phospholipase C-PI-PLC** was pre-prepared from culture medium of *Staphylococcus aureus* as described in detail previously (26, 27). The enzymatic preparation was employed the pooled and lyophilized fractions of the peak elute from Sephadex G-75 column, having a specific activity of 6000 units/mg of protein (27). The lyophilized enzyme was reconstituted in ice-cold distilled water and could be stored either at 0-2 °C or frozen in −20 °C for up to several weeks without appreciable loss of activity.

**Digestion of BBMS with PI-PLC** (Fig. 3)—BBMS (30-50 mg of protein) was suspended in 5 ml of a medium containing 60 mM sucrose, 50 mM Hepes/NaOH buffer (pH 7.4), and 2 μg of PI-PLC/tube, and preincubated for 60 min at 0-2 °C in order to allow the PI-PLC to equilibrate throughout the BBMS infoldings. This mixture was then incubated at 37 °C in a shaking water bath for 30 min. At the end, the mixture was cooled on ice and centrifuged at 100,000 × g for 60 min at 0-4 °C. A control BBMS suspension, without added PI-PLC, was treated simultaneously in the same manner. The 100,000 × g supernatants were collected and the BBMS pellets were resuspended in 4 ml of 300 mM mannitol-containing 5 mM Hepes, pH 8.5. Aliquots were then taken from the resuspended 100,000 × g pellets, and the 100,000 × g supernatants and frozen at −80 °C for further enzymatic assays and/or electrophoretic analysis. The resuspended BBMS pellets were then used for preparations of BBMV (Fig. 2).

**Electrophoresis**—Preparations of BBMS and BBMV with or without digestion with PI-PLC were analyzed by slab gel electrophoresis using a modification of the method of Littlefield (39). The 5% polyacrylamide gels (0.75 mm thick) containing 0.1% Triton X-100 were run in a vertical slab gel electrophoresis unit (Hoefler, SE 500). The samples to be analyzed were added to a buffer containing, at final concentration: 6.7 mm β-mercaptoethanol, 0.67% Triton X-100, 25% sucrose, 20 mM Tris, 150 mM glycine (pH 8.5), and 10 μg/ml of bromphenol blue as a tracking dye. These were mixed vigorously and then centrifuged at 7000 × g for 10 min in Beckman microfuge. Samples (10-25 μg of protein/well) were then subjected to electrophoresis at constant voltage (200 V) for about 2 h. The upper running buffer consisted of 20 mM Tris, 150 mM glycine, and 1% Triton X-100 (pH 8.5). The lower running buffer consisted of 5 mM Tris and 38 mM glycine (pH 8.5). Activity of alkaline phosphatase was visualized using Kaplow's histochemical staining procedure (40).

**Enzyme Assays**—Alkaline phosphatase was assayed at a pH of 10.5 with p-nitrophenyl phosphate as substrate as described previously (15). Leucine aminopeptidase (17, 31, 32), maltase (17, 33), and γ-glutamyltranspeptidase (34) were also assayed as described in the previous communications (15, 16, 31, 32, 33, 36). Protein content was determined by a modification of the Method of Lowry et al. (35) as described previously (15, 16, 36, 38).

All biochemicals and chemicals were obtained from the standard suppliers and were of the highest purity grades. Free acid 32P, L-[3H]proline, and D-[3H]glucose were purchased from New England Nuclear.

**RESULTS**

BBMV prepared from BBMS showed typical uphill transport uptake in the presence of a Na+(Na+, > Na+) gradient, reaching a peak in about 2 min, and then declining to a near-equilibrium state at 120 min (Fig. 1). As noted in most of the previous studies from our (16-18, 31) and other laboratories (3), complete equilibrium was not achieved for transport of 32P, even after 120 min, but was achieved in the transport of D-glucose and L-proline (31, 36). Compared to BBMV, BBMS did not show concentric uphill uptake of 32P above the equilibrium value although there was increased 32P uptake in the initial (0.5 min) phase in the presence of the Na+-gradient (Table I). This comparison indicates that concentric uptake of P, against an electrochemical P, gradient occurs only when the membranes are present in a soluble form. The results indicate that the BBMS used in the present study appear to be more aggregated and less permeable than the BBMV. Although a considerable difference was found between BBMS and BBMV in the uptake gradients of Na+-gradient, the results also indicate that the amount of Na+ transport in BBMV did not give a significant difference when compared to BBMS (Table II).

**TABLE I**

| Preparative step | Enzyme activities | 32P uptake |
|------------------|-------------------|------------|
|                  | Alkaline phosphatase | Leucine aminopeptidase | 0.5 min | 75 min | Δ% ratio | pmol/mg protein |
| Cortical homogenate | 1.5 ± 0.2 | 2.6 ± 0.3 | | | | |
| BBMS | 18.1 ± 7 (12 ×) | 19.7 ± 0.9 (7.5 ×) | NaCl | 529 ± 42 | 909 ± 27 | Δ = 13% |
|       | 12 (×) | 19.7 ± 0.9 (7.5 ×) | KCl | 123 ± 15 | 521 ± 45 | |
| BBMV | 34.7 ± 5.3 (21.5 ×) | 34.4 ± 8.4 (13.2 ×) | NaCl | 1398 ± 23 | 473 ± 9 | Δ = 195% |
|       | 23 (×) | 34.4 ± 8.4 (13.2 ×) | KCl | 85 ± 6.6 | 322 ± 7 | |

1. Ratio of Na+ gradient-dependent 32P uptake at uphill phase (0.5 min) and late (75 min) "equilibrium" phase of 32P transport.
2. Mean ± S.E. of 4 replicates. Values in parentheses represent increase of activity relative to homogenate.
3. NaCl uptake in the presence of Na+ gradient, KCl uptake in the presence of Na+ gradient (NaCl in medium replaced by KCl).

**TABLE II**

| Effect of incubation of BBMS with PI-PLC on brush-border enzymes | Alkaline phosphatase | Leucine aminopeptidase | γ-Glutamyltranspeptidase | Maltase |
|-------------------|-------------------|-------------------|-------------------|--------|
| BBMS before incubation | 33.4 ± 10.5 | 19.3 ± 5.5 | 61.7 ± 2.2 | 15.2 ± 1.1 |
| Control | 36.1 ± 11.7 | 4.9 ± 2.2 | 18.4 ± 0.8 | 16.6 ± 3.3 |
| PI-PLC-treated | 68.2 ± 3.6 | 68.7 ± 11 | 12.5 ± 0.6 | 12.2 ± 0.6 |
| BBMS after incubation | | | | |
| (a) 100,000 × g pellet | 36.1 ± 11.7 | 4.9 ± 2.2 | 18.4 ± 0.8 | 16.6 ± 3.3 |
| (b) 100,000 × g supernatant | 62.3 ± 2.0 | 68.7 ± 11 | 12.5 ± 0.6 | 12.2 ± 0.6 |
| BBMV prepared from BBMS | 71.2 ± 22.9 | 9.6 ± 14 | 135.0 ± 13.3 | 142 ± 10.7 |
| Control | 62.3 ± 2.0 | 68.7 ± 11 | 12.5 ± 0.6 | 12.2 ± 0.6 |
| PI-PLC-treated | 51.3 ± 0.5 | 4.9 ± 0.6 |

1. Values are mean ± S.E. from three experiments.
2. N.D., nondetectable activity.
Effect of alkaline phosphatase removal on brush-border transport of \(^{32}\text{P}_{\text{a}}\)-l-\[^{3}H\]proline, and d-\[^{3}H\]glucose

Na\(^{+}\)-dependent transport was measured in the presence of 150 mM \((\text{Na}^{+} > \text{K}^{+})\)-gradient; in Na\(^{+}\)-independent transport assay, NaCl was replaced by KCl. “Overshoot” is a ratio of “uphill” uptake at 0.5 min or 0.25 min to “equilibrium” uptake at 120 min, expressed as \(\%\) of equilibrium.

| Table III |

| Control | PI-PLC- treated | \(\Delta\%\) | Control | PI-PLC- treated | \(\Delta\%\) | Control | PI-PLC- treated | \(\Delta\%\) |
|---------|-----------------|-------------|---------|-----------------|-------------|---------|-----------------|-------------|
| Alkaline phosphatase | | | | | | | | | |
| (a) Specific activity (\(\mu\text{mol}\)/h/ mg protein) | 83.4 | 11.8 | -86% | 102.7 | 7.2 | -93% | 27.3 | 9.7 | -64% |
| (b) Total activity (\(\mu\text{mol}\)/h/ fraction) | 400.0 | 43.0 | -89% | 647.0 | 44.0 | -93% | 154.0 | 63.0 | -59% |
| P\(_{\text{i}}\) transport | | | | | | | | | |
| Na\(^{+}\)-dependent \(^{32}\text{P}_{\text{a}}\), uptake (\(\mu\text{mol}/\text{mg protein}\)) | 2037 \pm 110 | 2154 \pm 73 | 2071 \pm 37 | 2601 \pm 51 | 1611 \pm 48 | 1357 \pm 3 |
| “Overshoot” (\(\Delta\%\)) | +264% | +180% | +224% | +242% | +157% | +155 |
| Na\(^{+}\)-independent \(^{32}\text{P}_{\text{a}}\), uptake (\(\mu\text{mol}/\text{mg protein}\)) | 559 \pm 6 | 768 \pm 49 | 638 \pm 32 | 761 \pm 27 | 628 \pm 25 | 531 \pm 40 |
| \(\Delta\%\) | +231% | +177% | +224% | +242% | +157% | +155 |
| B-Glucose transport | | | | | | | | | |
| Na\(^{+}\)-independent uptake of d-\[^{3}H\]glucose (\(\mu\text{mol}\)/h/ mg protein) | 567 \pm 13 | 613 \pm 1 +37 | 555 \pm 16 | 486 \pm 9 |
| “Overshoot” (\(\Delta\%\)) | +111% | +77% | +46% | +56% | +111% | +112% |
| Na\(^{+}\)-dependent uptake of l-[\(^{3}H\)]proline (\(\mu\text{mol}\)/h/ mg protein) | 106 \pm 4 | 144 \pm 15 | 125 \pm 10 | 131 \pm 56 |
| “Overshoot” (\(\Delta\%\)) | +435% | +325% | +200% | +148% | +377 | +452 |

Comparison of P\(_{\text{i}}\) transport in BBMV prepared from kidneys of rabbits fed either high P diet or low P diet, with or without removal of alkaline phosphatase by PI-PLC treatment

Treatment with PI-PLC resulted in removal of 89 \(\pm\) 1% (high P diet) and 82 \(\pm\) 3% (low P diet) alkaline phosphatase (for details, see text). All values are mean \(\pm\) S.E.

| Table IV |

| Time (min) | High P diet | Low P diet |
|-----------|-------------|------------|
| Control | \(^{32}\text{P}_{\text{a}}\), uptake in the presence of NaCl | \(^{32}\text{P}_{\text{a}}\), uptake in the presence of KCl |
| 0.5 | 954 \pm 22 | 3420 \pm 273 |
| 120 | 632 \pm 26 | 660 \pm 24 |
| \(\Delta\%\) | +51% | +403% |
| \(^{32}\text{P}_{\text{a}}\), uptake in the presence of NaCl | \(^{32}\text{P}_{\text{a}}\), uptake in the presence of KCl |
| 0.5 | 74 \pm 12 | 97 \pm 13 |
| 120 | 371 \pm 32 | 345 \pm 48 |
| After deletion of alkaline phosphatase | \(^{32}\text{P}_{\text{a}}\), uptake in the presence of NaCl | \(^{32}\text{P}_{\text{a}}\), uptake in the presence of KCl |
| 0.5 | 1247 \pm 113 | 5441 \pm 132 |
| 120 | 832 \pm 44 | 926 \pm 114 |
| \(\Delta\%\) | +50% | +488% |
| \(^{32}\text{P}_{\text{a}}\), uptake in the presence of NaCl | \(^{32}\text{P}_{\text{a}}\), uptake in the presence of KCl |
| 0.5 | 116 \pm 45 | 97 \pm 2 |
| 120 | 602 \pm 102 | 439 \pm 7 |

\(\Delta\%\) increase of P\(_{\text{i}}\) uptake after 0.5 min relative to equilibrium value.

in the closed-compartment, sealed vesicular structure of the BBMV.

Incubation of BBMS with PI-PLC resulted in a selective removal, compared to other BBM enzymes, of alkaline phosphatase activity from BBMS (Table II). There was a marked (up to about 90\%) decrease in specific activity (Table II) and total activity (Tables II and III) of alkaline phosphatase in PI-PLC-treated BBMS. Alkaline phosphatase was not detectable in the 100,000 \(\times\) g supernatant of control BBMS preparation, but in the PI-PLC-treated preparation almost all activity of alkaline phosphatase was recovered in the 100,000 \(\times\) g supernatant. In contrast, there was no decrease in activity of leucine aminopeptidase or \(\gamma\)-glutamyltranspeptidase in BBMS pellets and no activity of these two enzymes was detected in 100,000 \(\times\) g supernatants (Table II). Also, the activity of maltase was not different between control and PI-PLC-treated BBMS. While maltase was partially released into the 100,000 \(\times\) g supernatant during incubation of BBMS at 37°C, but the extent of release was identical in the control and PI-PLC-treated BBMS.

Activity of alkaline phosphatase in the control BBMS preparation appeared as two distinct bands in the slab gel electrophoreogram (Tracks 1 and 2, Fig. 4), but in samples of PI-PLC-treated BBMS membranes (Tracks 3 and 4, Fig. 4), the alkaline phosphatase bands were decreased to nearly undetectable levels. Electrophoretic analysis of 100,000 \(\times\) g supernatant of incubated BBMS (Tracks 9 and 10, Fig. 4) showed just the opposite. While no activity of alkaline phosphatase activity was detected in supernatants of control BBMS (Track 9), two prominent alkaline phosphatase bands

![Fig. 4. Polyacrylamide gel electrophoresis of brush-border membrane preparations, control or digested with PI-PLC. Dark areas show location of alkaline phosphatase activity after specific staining. For the design of the experiment see Fig. 3 and “Methods.” Control BBMS, Tracks 1 and 2; BBMS after incubation with PI-PLC, Tracks 3 and 4. BBMV prepared from control BBMS, Tracks 5 and 6; BBMV prepared from BBMS incubated previously with PI-PLC, Tracks 7 and 8. 100,000 \(\times\) g supernatant from control BBMS, Track 9; 100,000 \(\times\) g supernatant from BBMS treated with PI-PLC, Track 10.](http://www.jbc.org/Downloaded from)
were found in the supernatant from PI-PLC-treated BBMS (Track 10, Fig. 4).

Transport properties of BBMV prepared from control BBMS were compared with those prepared from BBMS treated with PI-PLC (Table III). We measured the initial uphill uptake of $^{32}$P, D-[H]glucose, and L-[H]proline at 0.5 or 0.25 min and equilibrium point uptake (at 120 min) in the presence of the Na$^+$-gradient. $^{32}$P uptake was also measured in the absence of Na$^+$-gradient (Table III). Incubation of BBMS with PI-PLC removed most of (up to approximately 90%) the membrane-bound alkaline phosphatase but $^{32}$P transport by these alkaline phosphatase-depleted BBMV did not differ significantly from the transport seen in the controls. Na$^+$-gradient-dependent and independent uptake of $^{32}$P was similar in both preparations, as were uptakes of D-[H]glucose and L-[H]proline at the “uphill” phase and at “equilibrium.”

The extent of removal of alkaline phosphatase from BBMS was similar for BBMS prepared from kidneys of rabbits maintained on low P or high P diets (Table IV). The increased rate of $^{32}$P uptake observed in BBMV from rabbits fed the low P diet was not diminished by PI-PLC treatment. On the contrary, $^{32}$P uptake by BBMV was actually enhanced following treatment of BBMS with PI-PLC. The effect of PI-PLC treatment on properties of BBMV isolated from rabbits fed low P diet was then examined more closely in the next series of experiments (Table V). The rate of $^{32}$P uptake in the initial “uphill” phase was considerably increased in PI-PLC-treated BBMV, but neither $^{32}$P uptake at equilibrium point (120 min) nor $^{32}$P uptake in the absence of Na$^+$ was influenced by PI-PLC treatment. Although uptake of L-[H]proline seemed slightly higher in PI-PLC-treated BBMV, this difference was not a constant finding and did not reach statistical significance.

### DISCUSSION

In the present study, we examined the controversial question (1, 6–9) of whether alkaline phosphatase is directly related to Na$^+$-gradient-dependent transport of P, across the BBM. In the past, this question has been investigated mainly (a) by the observation of parallelism between changes in the rate of P transport and in the activity of alkaline phosphatase (1, 6, 7, 9–11, 13, 14, 16–19) or (b) with use of alkaline phosphatase inhibitors (1, 6, 8, 9, 23). As discussed in the introduction, these methodologies have major drawbacks and are not adequate to establish a direct causal nexus between the two parameters. To examine the role of alkaline phosphatase in BBM transport of P, in a more direct way, we took advantage of the recent finding that incubation of membranes with purified PI-PLC releases alkaline phosphatase from the membrane matrix (24, 25).

Our results show that incubation with PI-PLC indeed almost completely removed alkaline phosphatase from the matrix of BBM (Table II). Removal of alkaline phosphatase was very specific; the other BBM enzymes, e.g., γ-glutamyltranspeptidase and leucine aminopeptidase which constitute a greater portion of BBM matrix protein (37), were not even partially removed. The release of alkaline phosphatase from BBMS after incubation with PI-PLC was documented by measurement of enzymatic activity released into the soluble fraction (Table II) and was also confirmed by electrophoretic analysis (Fig. 4). The faster rate of electrophoretic migration of solubilized alkaline phosphatase (Fig. 4) also observed by others (25) suggests that alkaline phosphatase is not only detached from BBM by PI-PLC treatment, but also modified in a yet unknown way (25).

Our observations that BBMV depleted of alkaline phosphatase can transport $^{32}$P, D-[H]glucose, and L-[H]proline as effectively as control BBMV with intact alkaline phosphatase provides a strong and perhaps definitive argument against alkaline phosphatase being directly involved as an integral component of the BBM transport system for P, or of the transport system for D-glucose and L-proline within the same membrane.

Since a potent stimulus to increase $^{32}$P transport, in BBM, deprivation of dietary P (1, 4, 6, 7, 36), also increases alkaline phosphatase (15–17), we further examined whether alkaline phosphatase was involved in this adaptive phenomenon. We found that removal of alkaline phosphatase from BBM by PI-PLC treatment not only did not abolish the adaptive increase in P uptake by BBM (Table IV), but, unexpectedly, actually further enhanced the P uptake capacity (Table V). The PI-PLC-mediated effect was relatively specific for the Na$^+$-gradient-dependent uptake of $^{32}$P; uptake at the 120-min equilibrium point or Na$^+$-independent uptake of P, was not significantly affected. Uptake of L-proline at equilibrium was unaffected by the PI-PLC treatment which suggests that the enhanced P uptake was not due to a higher intravesicular volume. Further, the minimal effect on L-[H]proline transport (Table V) indicates that treatment with PI-PLC affected preferentially the transport system for P, or of alkaline phosphatase, or both, was responsible for the enhancement of P transport.

The relationship between BBM transport of P, and alkaline

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**TABLE V**

Effect of alkaline phosphatase removal on $^{32}$P uptake and L-[H]proline uptake by BBMV prepared from rabbits fed low P diet

|                      | In presence of NaCl | In presence of KCl |
|----------------------|---------------------|--------------------|
|                      | 0.5 min             | 120 min            | Δ% 0.5 min/120 min* | 0.5 min             | 120 min            | Δ% 0.5 min/120 min* |
| $^{32}$P uptake      |                     |                    |                    |                     |                    |
| Control              | 1892 ± 734          | 640 ± 80           | Δ +176 ± 33%       | 134 ± 37            | 357 ± 56           | Δ +325 ± 47%        |
| PI-PLC-treated       | 3572 ± 622a         | 887 ± 188          | Δ + 325 ± 47%*     | 147 ± 57            | 482 ± 72           | Δ +325 ± 47%*       |
| L-[H]Proline uptake |                     |                    |                    |                     |                    |
| Control              | 180 ± 25            | 76 ± 13            | Δ + 143 ± 13%      | 13% ± 25            | 13% ± 25           | Δ + 178 ± 35%       |
| PI-PLC-treated       | 305 ± 71            | 113 ± 23           | Δ + 143 ± 13%*     | 13% ± 25            | 13% ± 25           | Δ + 178 ± 35%*      |

* Increase of $^{32}$P uptake after 0.5/0.25 min relative to "equilibrium" value.

* Value significantly ($p < 0.05$ or higher degree of significance) different from corresponding controls; t-test.
FIG. 5. Schematic outline of possible relationships between alkaline phosphatase and the transport system for Pi within renal BBM. It is assumed that translocation of Pi across brush-border membrane may consist of several discrete steps in sequence (S, to S, from initial binding of Pi at the luminal surface (S,) up to the release of Pi into cytoplasm at the inner surface of BBM. AP denotes a molecule of alkaline phosphatase, it denotes a putative regulatory intracellular factor acting on BBM from the cell interior. Scheme A, alkaline phosphatase is an integral component of the BBM transport system, the target of an intracellular regulatory factor (R). Scheme B, alkaline phosphatase is not a component of the Pi transport system, but is located and related closely to the Pi transport system within BBM. Intracellular regulatory factor (R) influences Pi transport by acting first on alkaline phosphatase. Scheme C, alkaline phosphatase and the Pi transport system are both located within BBM and are influenced independently by an intracellular regulatory factor (R); both change in parallel without being related to each other. Scheme D, alkaline phosphatase is related closely to the Pi transport system within BBM. Regulatory factor (R) is acting on the Pi transport system; changes in Pi transport influence secondarily activity of alkaline phosphatase. Changed alkaline phosphatase can in turn modulate the Pi transport system.

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In conclusion, our results document that removal of alkaline phosphatase from renal BBM does not diminish the capacity for Na+ gradient-dependent transport of Pi. This was true under both normal dietary conditions and those where Pi transport was stimulated by dietary Pi deprivation. We suggest that alkaline phosphatase has no direct and essential role in Pi transport system within the BBM; however, it may modulate Pi transport in some as yet unknown manner.

The intracellular factor(s) which modulate the Pi transport system; changes in Pi transport influence secondarily activity of alkaline phosphatase. Changed alkaline phosphatase can in turn modulate the Pi transport system.
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