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Different molecular sizes for Na^+-dependent phosphonoformic acid binding and phosphate transport in renal brush border membrane vesicles

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We compared several features of Na^+-dependent phosphono[^{14}C]formic acid (PFA) binding and Na^+-dependent phosphate transport in rat renal brush border membrane vesicles. From kinetic analyses, we estimated an apparent K_m for PFA binding of 0.86 mM, an order of magnitude greater than that for phosphate and the high-affinity phosphate transport system. A hyperbolic Na^+-saturation curve for PFA binding and a sigmoidal Na^+-saturation curve for phosphate transport were demonstrated; based on these data, we estimated stoichiometries of 1:1 for Na^+/PFA and 2:1 for Na^+/phosphate. By radiation inactivation analysis, target sizes for brush border membrane protein(s) mediating Na^+-dependent PFA binding and Na^+-dependent phosphate transport corresponded to molecular masses of 555 ± 32 kDa and 205 ± 36 kDa, respectively. Similar analysis of the phosphate-inhibitable component of Na^+-dependent PFA binding gave a target size of 130 ± 28 kDa. We also demonstrated that phosphate deprivation, which elicits a 2.6-fold increase in brush border membrane Na^+-dependent phosphate transport, had no effect on either Na^+-dependent PFA binding or on the target size for PFA binding. However, phosphate deprivation appeared to increase the target size for phosphate transport (from 255 ± 32 to 335 ± 75 kDa ($P < 0.01$)). In summary, we present evidence for several differences between Na^+-dependent PFA binding and Na^+-dependent phosphate transport in rat renal brush border membrane vesicles and suggest that PFA may not interact exclusively with the proteins mediating Na^+-phosphate co-transport.

Introduction

It is well established that phosphate is reabsorbed from the glomerular filtrate via a Na^+-gradient dependent mechanism at the brush border membrane of proximal tubular cells [1,2]. Many indirect approaches have been used to obtain information about the molecular aspect of the phosphate carrier, including chemical modification [3,4] purification and reconstitution [5,6], and radiation inactivation of membrane proteins [7]. In spite of these efforts, the identity of the proteins mediating renal brush border membrane phosphate transport remains unknown.

Recent studies have demonstrated that phosphonocarboxylic acids are potent and selective inhibitors of renal brush border membrane phosphate transport [8]. Phosphonoformic acid (PFA) inhibits both the high- and low-affinity phosphate transport processes of mouse renal brush border membranes in a competitive fashion [9] and unlike other inhibitors of phosphate transport, PFA has no effect on brush border membrane alkaline phosphatase or basolateral membrane Na^+,K^+-ATPase [8]. Szczepanska-Konkel et al. reported that ^{14}C-PFA binds specifically to the luminal surface of renal brush border membranes [10]. The binding is Na^+-dependent and inhibitable by PFA and phosphate but not by diphosphonates, glucose or proline [10]. On the basis of these results it was proposed that PFA is a useful probe

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for studies of the brush border membrane phosphate transporter, in a manner similar to phloridzin, which specifically binds to the Na^+-glucose co-transporter of brush border membranes [11].

The present study was undertaken to compare the target sizes for Na^+-dependent ^{14}C-PFA binding and phosphate transport in rat renal brush border membrane vesicles by radiation inactivation analysis. In addition, we examined whether dietary phosphate deprivation which is known to elicit an adaptive increase in brush border membrane Na^+-dependent phosphate transport [12], has an effect on Na^+-dependent PFA binding and on the radiation inactivation sizes (RIS) for PFA binding and phosphate transport.

Materials and Methods

Membrane preparation

Adult male Sprague Dawley rats, 250–300 g, were fed a standard Purina diet (No. 5001) containing 0.8% phosphate and 2% calcium. To examine the effect of phosphate deprivation, rats were fed either low phosphate or control diets containing 0.03% phosphate (Teklad 86128) and 1% phosphate (Teklad 86129), respectively, for 4 days. The diets contained 1% calcium and were identical in all other respects. After 4 days, the rats were killed by decapitation and brush border membranes purified by $MgCl_2$ precipitation [13]. The brush border membranes were washed and resuspended in a cryoprotective medium containing 150 mM KCl, 5 mM Tris-Hepes (pH 7.5), 14% glycerol, 1.4% sorbitol and stored in liquid nitrogen until use. Alkaline phosphatase enrichment of brush border membrane preparations was routinely 11.3 ± 0.9-fold (mean ± S.E., $n = 6$).

Irradiation procedure

Irradiation was performed in a Gammacell Model 220 instrument at a dose rate of approximately 1.5 Mrad/h. During this procedure the samples were maintained in dry ice. Controls were run concurrently under the same conditions but without irradiation, as described previously [7]. The empirical Eqn. 1 was used to relate radiation inactivation size (RIS) to $D_{37,t}$, the radiation dose (in Mrad) necessary to inactivate transporters or binding proteins to 37% of their initial value, and to t, the temperature (in °C) [14].

$$\log \text{RIS} = 5.89 - \log D_{37,t} - 0.0028t \quad (1)$$

$D_{37,t}$ values were obtained from a semi-logarithmic plot of uptake or binding versus irradiation dose using a least-square fit. The experimental values obtained from transport activity were corrected for intravesicular volume as measured by glucose equilibrium [15].

Phosphate transport

Phosphate uptake was performed in quadruplicate at 25 °C by the addition of 80–120 μg of protein to incubation media containing 5 mM Tris-Hepes buffer (pH 7.5), 14% glycerol, 1.4% sorbitol, 150 mM NaCl or 150 mM KCl, and 200 μM [^{32}P]phosphate (3 μCi). After different times of incubation, the reaction was stopped by dilution (1/30) with an ice-cold stop solution (5 mM Tris-Hepes (pH 7.5), 14% glycerol, 1.4% sorbitol and 150 mM KCl). The suspension was filtered immediately under vacuum through a cellulose filter (0.45 μm). The filter was rinsed with 8 ml of stop solution and the radioactivity counted.

PFA binding

^{14}C-PFA binding was performed in quadruplicate at 25 °C by addition of 80–120 μg of protein to incubation media containing, 5 mM Tris-Hepes (pH 7.5), 14% glycerol, 1.4% sorbitol, 810 μM ^{14}C-PFA (0.2 μCi) and 150 mM NaCl or KCl. Incubation time was fixed at 30 min unless otherwise indicated, and the reaction was stopped by dilution (1/60) with an ice-cold stop solution. The stop solution was the same as the incubation medium but without PFA. After filtration under vacuum, the filters were rinsed with 6 ml of stop solution and processed for liquid scintillation counting. For the measurement of phosphate-displaceable PFA binding, brush border membrane vesicles were equilibrated, before irradiation, in 150 mM NaCl, 14% glycerol, 1.4% sorbitol and 5 mM Tris-Hepes (pH 7.5), with or without 20 mM phosphate, as described by Hoppe et al. [16]. Binding of PFA was measured in an incubation medium containing 810 μM-^{14}C-PFA, 150 mM NaCl, 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5), in the presence or absence of 20 mM phosphate. Phosphate-displaceable PFA binding was calculated as the difference between PFA binding measured in the absence and presence of 20 mM phosphate as described by Hoppe et al. [16].

Statistical methods

Statistical analysis (Student's t-test) was performed using a computer statistical package (Statview).

Chemicals

[^{32}P]Orthophosphate (carrier free) was purchased from Dupont-New England Nuclear and phosphono-[^{14}C]formic acid, tripotassium salt, (24.8 mCi/mmol) from Amersham Canada Ltd. Other chemicals were of the highest available purity.

Results

Characteristics of ^{14}C-PFA binding

The Na^+-stimulated component of ^{14}C-PFA binding to rat renal brush border membrane vesicles was tem-

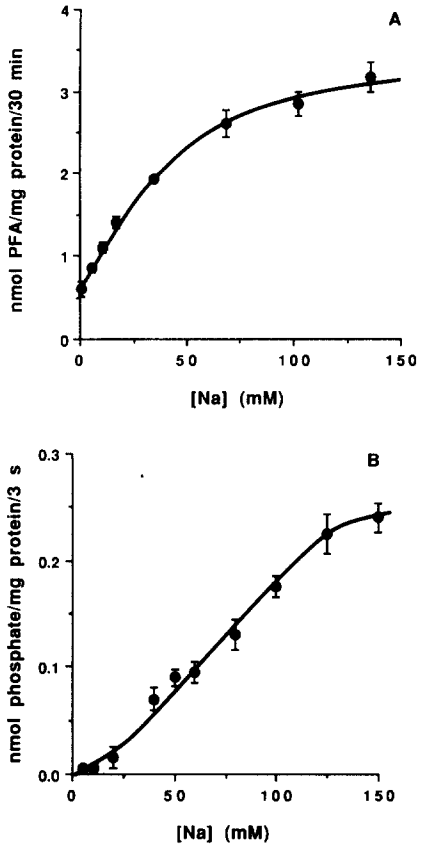

Fig. 1. Effects of extravesicular Na^+ concentration on ^{14}C-PFA binding and [^{32}P]phosphate uptake. (A) PFA binding was measured at 30 min in incubation media containing 810 μM ^{14}C-PFA (0.2 μCi), 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5) over a concentration range of 0 to 140 mM NaCl. Total osmolarity was adjusted to 300 mosM with KCl. (B) Phosphate transport was measured at 3 s with the same media except that PFA was replaced with 200 μM [^{32}P]phosphate and NaCl with $NaNO_3$. Each value represents the mean ± S.E. of three experiments each done in quadruplicate.

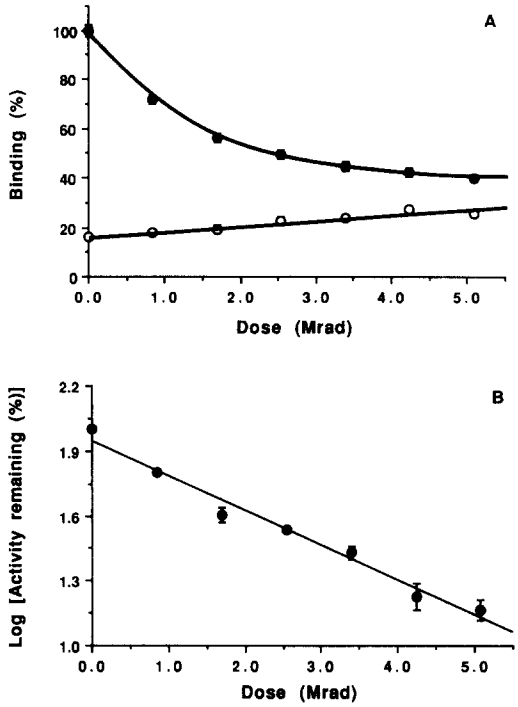

Fig. 2. Molecular size determination of the PFA binding complex. (A) Binding of PFA was measured at 30 min in incubation media containing 810 μM ^{14}C-PFA (0.2 μCi), 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5) and 150 mM NaCl (●) or 150 mM KCl (○). 100% activity represents 4.02 ± 0.14 and 0.67 ± 0.07 nmol/mg protein per 30 min for binding measured in the presence of NaCl and KCl, respectively. (B) Na^+-dependent PFA binding was calculated as the difference between binding measured in media containing NaCl and KCl. The results are expressed as log of the percentage of the remaining activity. Each value represents the mean ± S.E. of data derived from three experiments each done in quadruplicate.

perature dependent, saturable (K_m = 0.86 mM), and reached a steady state within 30 min of incubation at 25 °C (data not shown), consistent with the findings of Szczepanska-Konkel et al. [10].

The effects of increasing concentrations of extravesicular Na^+ on ^{14}C-PFA binding (Fig. 1A) and phosphate transport (Fig. 1B) were compared. A hyperbolic relationship between Na^+ concentration and PFA binding was observed, indicating a Na^+/PFA stoichiometry of 1 : 1. In contrast, a sigmoidal relationship between Na^+ concentration and phosphate transport was obtained indicating that more than one Na^+ was interacting with the phosphate transport system; a Na^+/phosphate stoichiometry of 1.74, estimated by a Hill plot, is in agreement with that reported previously [2,3]. The apparent K_{Na} for PFA binding, estimated by a Lineweaver-Burk plot, was 28 mM and for phosphate-uptake, estimated by a Hill plot, was 80 mM.

TABLE I

Effect of diet on radiation inactivation size for Na^+-dependent PFA binding and Na^+-dependent phosphate transport in rat renal brush border membrane vesicles

RIS values were calculated from the data shown in Figs. 2–6 using rat renal brush border membrane vesicles as described in Materials and Methods. The correction was made on the basis of a small reduction in intravesicular volume, measured by D-[^{14}C]glucose uptake at equilibrium before the irradiation, as described elsewhere [15]. Each value represents the mean ± S.D. of data obtained from 3–8 experiments.

	Radiation inactivation size (kDa)		
	standard Purina diet	control phosphate diet	low phosphate diet
Na^+-depending PFA binding	555 ± 32	667 ± 110	747 ± 113 [a]
Na^+-dependent phosphate transport	234 ± 42	282 ± 35	367 ± 82 [a,b]
Corrected	205 ± 36	255 ± 32 [a]	335 ± 75 [a,b]
PFA/P_i	2.7	2.6	2.2

[a] Significantly different from Standard Purina, $P < 0.025$.
[b] Significantly different from Control diet, $P < 0.05$.

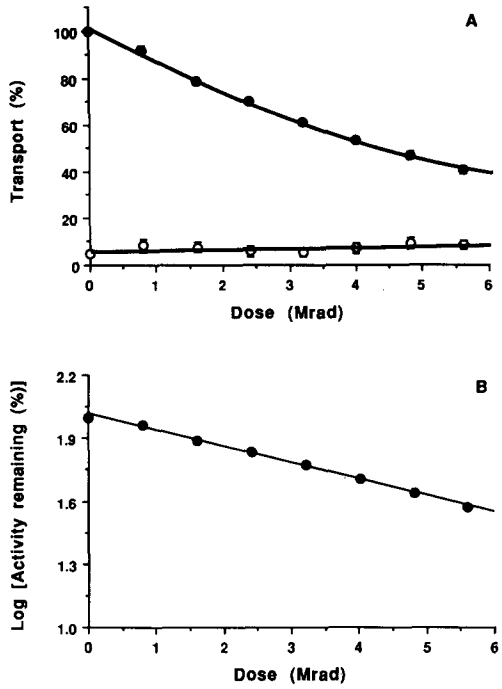

Fig. 3. Molecular size determination of the phosphate carrier. (A) Phosphate uptake was measured at 5 s in incubation media containing 200 μM [^{32}P]phosphate (3 μCi), 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5) and 150 mM NaCl (●) or 150 mM KCl (○). 100% activity represents 1.25 ± 0.07 and 0.068 ± 0.012 nmol/mg protein per 5 s for transport measured in the presence of NaCl and KCl, respectively. (B) Na^+-dependent phosphate transport was calculated as the difference between the uptakes measured in media containing NaCl and KCl. The results are expressed as log of the percentage of remaining activity. Each value represents the mean ± S.E. of data derived from six experiments each done in quadruplicate.

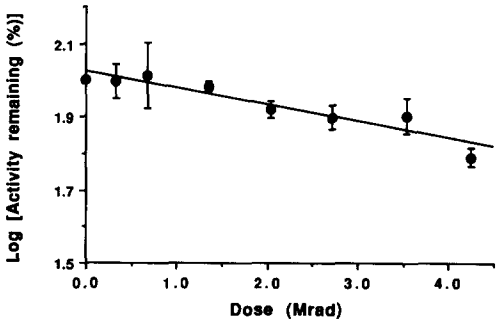

Fig. 4. Molecular size determination of the phosphate-displaceable PFA binding complex. Brush border membrane vesicles were equilibrated in 150 mM NaCl, 14% glycerol, 1.4% sorbitol and 5 mM Tris-Hepes (pH 7.5) with or without 20 mM phosphate before irradiation. Binding of PFA was measured in an incubation medium containing 810 μM ^{14}C-PFA, 150 mM NaCl, 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5) in the presence or absence of 20 mM phosphate. Phosphate-displaceable PFA binding was calculated as the difference between the binding of PFA measured in the absence and presence of phosphate. 100% activity represents 0.947 ± 0.13 nmol/mg protein per 30 min. The results are expressed as the log of the percentage of remaining activity. Each value represents the mean ± S.E. of the data derived from four experiments each done in quadruplicate.

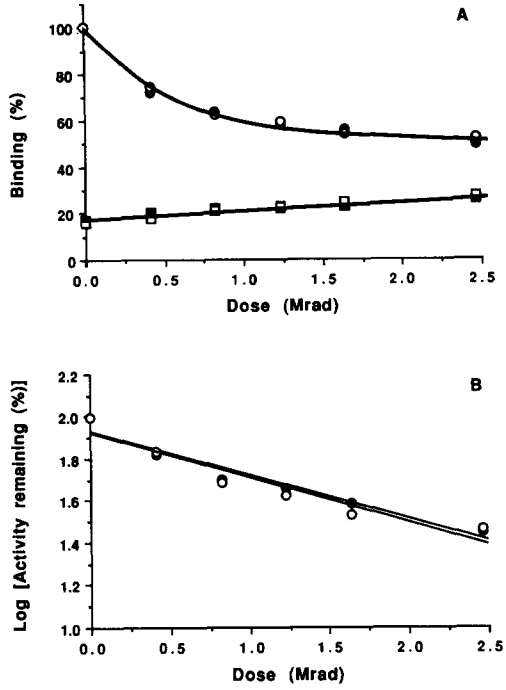

Fig. 5. Effect of low phosphate diet on molecular size of PFA binding complex. (A) Binding of PFA to brush border membrane vesicles isolated from rats fed control (●, ■) or low phosphate diets (○, □) was measured at 30 min in incubation media containing 810 μM ^{14}C-PFA (0.2 μCi), 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5) and 150 mM NaCl (●, ○) or 150 mM KCl (■, □). 100% activity represents 4.39 ± 0.13 and 0.68 ± 0.04 nmol/mg protein per 30 min (control diet) and 4.02 ± 0.14 and 0.67 ± 0.07 nmol/mg protein per 30 min (low phosphate diet) for binding measured in the presence of NaCl and KCl, respectively). (B) Na^+-dependent PFA binding was calculated as the difference between binding measured in media containing NaCl and KCl. The results are expressed as log of the percentage of the remaining activity. Each value represents the mean ± S.E. of data derived from at least six experiments each done in qudruplicate.

RIS for Na^+-dependent PFA binding

The molecular size of the brush border membrane protein(s) involved in Na^+-dependent PFA binding was determined by radiation inactivation analysis. In the presence of an Na^+-gradient there was a progressive loss of binding activity (Fig. 2A). In contrast, Na^+-independent PFA binding was not affected by these doses of irradiation. The radiation inactivation size, estimated from a semi-logarithmic plot of the Na^+-dependent component (Na-K) (Fig. 2B), was 555 ± 32 kDa. The RIS for Na^+-dependent phosphate transport determined in the same brush border membrane preparations was 234 ± 42 kDa (Fig. 3A and 3B), a value identical to that reported previously [7] and significantly less than the target size for Na^+-dependent PFA binding (Table I). A small correction for the loss of intravesicular volume during the irradiation procedure, necessary when RIS for carrier proteins is calculated [15], was applied to phosphate transport inactivation size (Table I).

The RIS for the phosphate-inhibitable component of Na^+-dependent PFA binding was determined under Na^+-equilibrium conditions as described by Hoppe et al. [16]. A semilogarithmic plot of phosphate-inhibitable PFA binding as a function of radiation dose is depicted in Fig. 4. These data gave an RIS of 130 ± 28 kDa, a value significantly lower than that for either Na^+-dependent PFA binding or Na^+-dependent phosphate transport (Table I).

Effect of phosphate deprivation

Phosphate-deprivation led to a significant fall in plasma phosphate, a 2.6-fold increase in renal brush border membrane Na^+-dependent phosphate transport and no significant change in Na^+-dependent ^{14}C-PFA binding in renal brush border membrane vesicles (Table II).

The effect of dietary phosphate on target sizes for Na^+-dependent PFA binding and Na^+-dependent phosphate transport were also examined. Phosphate deprivation did not significantly alter RIS for Na^+-dependent PFA binding (Fig. 5A and 5B, Table I), although RIS for membranes from rats fed the control and low phosphate diets were higher than those for membranes from rats fed standard Purina Chow (Table I). Phosphate deprivation led to a small but significant increase in RIS for Na^+-dependent phosphate transport (Figs. 6A and 6B, Table I). Again, the sizes obtained for phosphate transport were higher in membranes from rats fed control and low phosphate diets than from those fed standard Purina Chow (Table I).

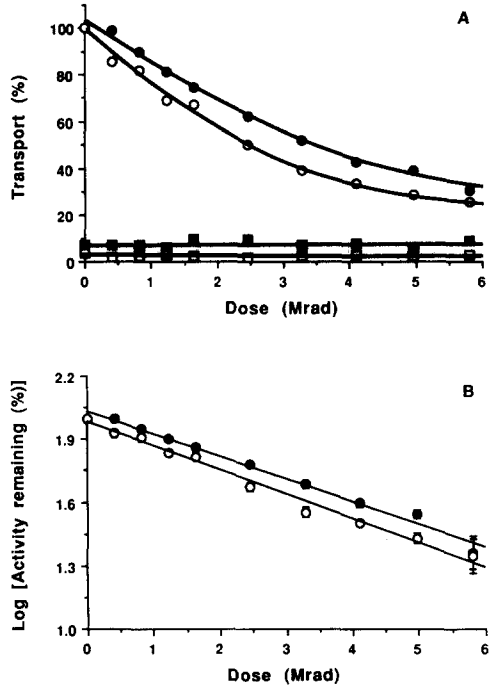

Fig. 6. Effect of low phosphate diet on molecular size of the phosphate carrier. (A) Phosphate uptake in brush border membrane vesicles isolated from rats fed control (●, ■) or low phosphate diet (○, □) was measured at 5 s in incubation media containing 200 μM [^{32}P]phosphate (3 μCi), 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 7.5) and 150 mM NaCl (●, ○) or 150 mM KCl (■, □). 100% activity represents 2.39 ± 0.15 and 0.16 ± 0.05 nmol/mg protein per 5 s (low diet) and 0.92 ± 0.05 and 0.12 ± 0.03 nmol/mg protein per 5 s (control phosphate diet) for transport measured in the presence of NaCl or KCl, respectively. (B) Na^+-dependent phosphate transport was calculated as the difference between the uptakes measured in media containing NaCl and KCl. The results are expressed as log of the percentage of the remaining activity. Each value represents the mean ± S.E. of data derived from at least seven experiments each done in quadruplicate.

TABLE II

Effect of the low phosphate diet on plasma phosphate and on Na^+-dependent phosphate transport and Na^+-dependent PFA binding in rat renal brush border membranes

Plasma phosphate data represent mean ± S.E. of 15 rats. Brush border membrane vesicles were prepared from rats fed the control and low phosphate diets as described in Materials and Methods. Vesicles were incubated for 5 s in a medium containing 200 μM [^{32}P]phosphate (3 μCi), 14% glycerol, 1.4% sorbitol, 5 mM Tris-Hepes (pH 75) and 150 mM NaCl or 150 mM KCl for phosphate uptake. PFA binding was determined under the same conditions with 810 μM ^{14}C-PFA (0.2 μCi) for 30 min. In both experiments the activity measured in KCl was subtracted from the activity measured in NaCl. Each value represents the mean ± S.E. of data obtained from four experiments for phosphate uptake and seven experiments for PFA binding, each done in quadruplicate.

	Control diet	Low phosphate diet
Plasma phosphate (mM)	2.43 ± 0.03	1.85 ± 0.04 [a]
Phosphate transport (nmol/mg protein per 5 s)	0.92 ± 0.05	2.39 ± 0.15 [a]
PFA binding (nmol/mg protein per 30 min)	4.02 ± 0.14	4.39 ± 0.13

[a] $P < 0.01$.

Discussion

It was recently suggested that PFA, a competitive inhibitor of Na^+-dependent phosphate transport at the renal brush border membrane [8,9], is a useful probe to study the Na^+-phosphate co-transporter [10]. In the present study, we compared several characteristics of Na^+-dependent PFA binding and Na^+-dependent phosphate transport in rat renal brush border membrane vesicles and provide evidence for differences between these two processes. In addition, we demonstrate that phosphate deprivation, which elicits an adaptive increase in brush border membrane Na^+-dependent phosphate transport, has no significant effect on Na^+-dependent PFA binding, thereby, confirming the results of a recently published study [17]. We also show that whereas phosphate deprivation does not significantly alter the target size for Na^+-dependent PFA binding, the RIS for Na^+-dependent phosphate transport appears to be significantly increased.

Characteristics of ^{14}C-PFA binding

Our kinetic studies demonstrate that the Na^+-dependent component of PFA binding is saturable, with an apparent K_m of approx. 1 mM. Several groups [9,18,19] have provided evidence for two kinetically distinct Na^+-phosphate co-transporters in renal brush border membranes with apparent K_m values of approx. 90 μM and 1.28 mM, respectively. Although, it is not immediately clear which of these transporters is binding PFA, the concentrations of PFA required to inhibit the high- and low-affinity phosphate transport processes ($K_i = 0.3$ mM and 9 mM, respectively [9]) suggest that under our assay conditions, PFA is binding to the high-affinity phosphate transport site. In the present study we also compared the Na^+-dependence of PFA binding and phosphate transport. We report that the K_{Na} for PFA binding (28 mM) is significantly lower than the K_{Na} for phosphate transport (80 mM). Moreover, we demonstrate a lack of correspondence in the Na^+-saturation curves for PFA binding and phosphate transport. The PFA binding curve is clearly hyperbolic, indicating a stoichiometry of 1 Na^+ to 1 PFA whereas phosphate transport observes a classical sigmoidal relationship with an estimated stoichiometry of 2 Na^+ to 1 phosphate, in agreement with results obtained by the direct method of measuring coupled fluxes [3].

Molecular sizes of PFA binding and phosphate transport activities

Radiation inactivation was used to compare the molecular size of the brush border membrane proteins involved in PFA binding and phosphate transport. As reported previously for Na^+-dependent phosphate transport [7], Na^+-dependent PFA binding was highly sensitive to low doses of irradiation in the presence of a Na^+-gradient. Since irradiation has no effect on lipids and sugars [14], these findings provide direct evidence that Na^+-dependent PFA binding, like Na^+-dependent phosphate transport [7], is protein-mediated. In contrast, neither PFA binding nor phosphate transport, when measured in the presence of a K^+ gradient, is affected by the same dose range of irradiation. These data suggest that both functions measured in K^+ are either non protein-mediated or that the proteins involved have a very low molecular size. The calculated RIS for Na^+-dependent PFA binding is 555 ± 32 kDa, a value which is 2.7-fold greater than that for Na^+-dependent phosphate transport. The RIS for the Na^+-phosphate co-transporter reported here (234 ± 42 kDa uncorrected; 205 ± 36 kDa corrected) is similar to that reported previously [7,15]. In the present study, we also demonstrate that the phosphate-displaceable component of Na^+-dependent PFA binding has a molecular size of 130 ± 28 kDa. This value is identical to that recently reported by Pierce [20] for the Na^+-phosphate co-transporter of the intestinal brush border membrane.

The precise relationship between the two PFA binding entities (555 kDa and 130 kDa) and the Na^+-phosphate co-transporter (234 kDa) is not clear. We suggested that Na^+-solute co-transporters in renal brush border membranes function as polymeric protein complexes [15,21] and support for this hypothesis was recently provided by Stevens et al. who demonstrated that the intestinal brush border membrane Na^+-glucose co-transporter functions in situ as a homotetramer [22]. The results presented here could be explained by such a hypothesis. Based on the present results and the similarity between the molecular sizes of the phosphate-displaceable component of PFA binding and the intestinal brush border membrane phosphate transporter [20], we suggest that the functional Na^+-phosphate co-transporter of renal brush border membranes is comprised of two 130 kDa monomers.

Alternatively, the 130 kDa phosphate-inhibitable PFA binding component may not be related to the renal brush border membrane Na^+-phosphate co-transporter. Tenenhouse and Lee recently demonstrated that (1) sulfate, which does not inhibit Na^+-dependent phosphate transport, is a potent inhibitor of Na^+-dependent PFA binding, (2) the inhibition of Na^+-dependent PFA binding by sulfate and phosphate is not additive, indicating that sulfate and phosphate are interacting with the same PFA binding sites and (3) PFA inhibits Na^+-dependent sulfate transport as well as Na^+-dependent phosphate transport in renal brush border membranes [23]. These results question the specificity of PFA binding to the renal brush border membrane Na^+-phosphate co-transporter and suggest that PFA may not be binding exclusively to the Na^+-phosphate co-transporter [23].

Effect of phosphate deprivation

In agreement with previous reports, phosphate deprivation elicited a significant increase in brush border membrane Na^+-dependent phosphate transport [2,12]. In contrast, PFA binding was not significantly altered by the low phosphate diet. These data suggest that either the adaptive increase in phosphate transport occurs by a mechanism not involving an increase in the number of transport sites [17] or that PFA binding may not reflect the number of phosphate transport sites [23] as discussed above.

In addition, our data demonstrate that while phosphate deprivation does not influence the RIS for Na^+-dependent PFA binding, it significantly increases the molecular size for Na^+-dependent phosphate transport. The low phosphate diet may elicit a redistribution of functional monomers with different intrinsic activities. The participation of different subunits or domains in calcium channel activity was similarly proposed by Catterall et al. [24]. These authors demonstrated different target sizes, determined by radiation inactivation analysis, of the calcium channel of skeletal muscle, using

different calcium antagonists, all of which bound to the channel with high affinity [24].

We are unable to explain why the RIS for both Na^+-dependent phosphate transport and Na^+-dependent PFA binding is higher in membranes isolated from rats fed the Teklad diets (control and low phosphate) than in membranes from rats fed the Purina diet. Others have also shown that diet can influence the relative proportion of two functionally active forms of rat liver microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase [25]. Although more work will be required to elucidate the basis for the diet-induced differences in target sizes for both PFA binding and phosphate transport in brush border membranes from rats, it should be noted that diet did not significantly influence the PFA binding/phosphate transport RIS ratio (Table I).

In summary, the present study demonstrates differences in the kinetics, Na^+-stoichiometry and RIS for Na^+-dependent PFA binding and Na^+-dependent phosphate transport in rat renal brush border membranes. These results suggest that transport function may be mediated by a dimeric membrane protein complex, consisting of subunits involved in phosphate-displaceable PFA binding. Alternatively, PFA may not be interacting exclusively with the renal Na^+-dependent phosphate transporter. Clearly further study will be required to assess the nature of the PFA interaction with the apical phosphate carrier in mammalian kidney.

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