Phosphate transport by rat intestinal basolateral-membrane vesicles

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The characteristics of phosphate transport across intestinal basolateral membranes of the rat were determined by using enriched preparations in which uphill Na^+ -dependent D-glucose transport could not be demonstrated, but ATP-dependent Ca^{2+} transport was present. Phosphate transport was saturable, Na^+ -dependent and exhibited Michaelis–Menten kinetics. V_{max} was 51.1 ± 4.2 pmol/10 s per mg of protein and K_m was 14 ± 3.9 μM . The transport process was electroneutral. Tracer-exchange experiments and counter-transport studies confirmed the presence of a $\text{Na}^+ - \text{P}_i$ carrier at the basolateral membrane. The presence of inside-positive membrane potential did not enhance phosphate uptake, indicating that the Na^+ effect is secondary to the presence of the $\text{Na}^+ - \text{P}_i$ carrier rather than an induction of positive membrane potential. The stoichiometry of this carrier at pH 7.4 was 2 $\text{Na}^+ : 1$ phosphate, as shown by direct studies utilizing the static-head method. These studies are the first to determine the presence of a phosphate carrier at the basolateral membrane.

INTRODUCTION

The metabolic function of the cell is dependent on the availability of intracellular P_i . We and others have characterized the process by which P_i enters the brush-border membranes of the intestinal epithelial cell. An electroneutral $\text{Na}^+ - \text{P}_i$ co-transport system has been described for rat and rabbit intestinal epithelial cells (Berner *et al.*, 1976; Danisi & Straub, 1980; Borowitz & Ghishan, 1985). A similar $\text{Na}^+ - \text{P}_i$ transport system has also been described in renal brush-border-membrane vesicles (Cheng & Sacktor, 1981). The mechanisms by which P_i is transported across the basolateral membranes of the intestinal epithelial cells have not been defined. A Na^+ -gradient-dependent P_i transporter has been characterized in basolateral membrane vesicles from dog kidney (Schwab *et al.*, 1984). Similarly Na^+ -gradient-dependent transporters for L-glutamate and L-glutamine have been described in basolateral-membrane vesicles from rabbit and dog kidney (Sacktor *et al.*, 1981; Windus *et al.*, 1984). On the other hand, Mircheff *et al.* (1980) demonstrated a Na^+ -dependent and a Na^+ -independent transport of alanine across the intestinal basolateral-membrane vesicles. The current study was designed to examine P_i transport by rat basolateral-membrane vesicles. We have utilized basolateral-membrane vesicles prepared by using a Percoll density gradient, in which Na^+ -dependent D-glucose transport, a characteristic of brush-border-membrane vesicles, could not be measured, whereas ATP-dependent Ca^{2+} uptake, a characteristic of basolateral membrane vesicles, is demonstrated.

MATERIALS AND METHODS

Materials

D- $[^3\text{H}]$ glucose (18.1 Ci/mmol) and $[^{32}\text{P}]\text{P}_i$ (carrier-free) were obtained from New England Nuclear Corp., Boston, MA, U.S.A. Valinomycin, enzymes and substrates for leucine aminopeptidase were obtained from

Sigma Chemical Corp., St. Louis, MO, U.S.A. Cellulose nitrate filters (0.45 μm pore size) were obtained from Sartorius Filters, Haywood, CA, U.S.A. All other chemicals were of the highest purity available.

Preparation of basolateral-membrane vesicles

Jejunal basolateral-membrane vesicles were prepared by using a modified centrifugation technique, followed by separation on a Percoll gradient (Scalera *et al.*, 1981). Two adolescent-rat jejunums were used for each preparation. The intestinal segments were removed, flushed with ice-cold Ringer's lactate solution (Buffer I) and then filled with warmed buffer solution (37 °C) containing 1.5 mM-KCl, 96 mM-NaCl, 8 mM- KH_2PO_4 , 5.6 mM- Na_2HPO_4 , 27 mM-sodium citrate and 2 mM-dithiothreitol (pH 7.4) (Buffer II). The segments were then clamped and incubated for 15 min in a shaking water bath at 37 °C. The clamps were then removed and the contents were emptied. The segments were then filled with ice-cold buffer containing 100 mM-mannitol, 100 mM-KCl and 24 mM-Hepes/Tris buffer, pH 7.4 (Buffer III), and gently palpated with fingers for 5 min to release epithelial cells. The contents were then drained into a beaker on ice, and the volume was made up to 250 ml with Buffer III. The cells were then centrifuged at 200 g for 5 min, and the cell pellet was homogenized in 250 ml of Buffer III in an Omni-mixer for 30 min. The homogenate was then made up to 300 ml with Buffer III, and centrifuged at 2500 g for 20 min. The supernatant was then collected and centrifuged at 22000 g for 25 min. The supernatant was discarded, and the resulting fluffy layer of the pellet was resuspended in 90 ml of Buffer III and homogenized in a glass/Teflon homogenizer (20 strokes). The resultant homogenate was mixed with Percoll (Pharmacia; 15%) and then centrifuged at 48000 g for 45 min. A distinct band of basolateral membranes was seen at the upper one-third of the Percoll gradient. The band was aspirated with a needle, and the pellet resuspended in the appropriate trans-

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port buffer. The resuspended pellet was then centrifuged at 48000 *g* for 20 min and finally suspended in 100 mM-KCl/100 mM-mannitol/10 mM-Hepes/Tris buffer (pH 7.4).

Transport measurements

Uptake of substrates (D-glucose, phosphate) was measured by a rapid-filtration technique (Hopfer *et al.*, 1973). All experiments were performed at 25 °C. Transport was initiated by adding 20 μ l of the final vesicle suspension to the desired incubation medium containing labelled substrate. The composition of the incubation medium for each individual experiment is described in the Table legends in the Results section. At the desired time intervals, the reaction was stopped by the addition of ice-cold stop solution, consisting of 100 mM-mannitol, 100 mM-NaCl, 20 mM-Hepes/Tris (pH 7.4) and 10 mM-K₃PO₄. For glucose studies, 0.5 mM-phloretin was also added. The vesicles were immediately collected on a cellulose nitrate filter (0.45 μ m pore size; Sartorius Filters) and kept under suction while being washed with ice-cold stop solution. The amount of radioactive substrate remaining on the filter was determined in a Beckman liquid-scintillation counter, with Bray's solution (New England Nuclear Corp., Boston, MA, U.S.A.) as the liquid scintillant. Radioactivity remaining in the filters after pipetting incubation medium into the radioactive substrate in the absence of vesicles was used as background and was considered in the calculations.

Purity of vesicle preparation

Purity of the basolateral-membrane preparations was determined by marker-enzyme activities. Leucine aminopeptidase, a marker for brush-border membranes, was measured with a kit from Sigma. Disaccharidases, another marker for brush-border membrane, were measured by the method of Dahlqvist (1964). Na⁺ + K⁺-dependent ATPase, a marker for basolateral membranes, was measured by the method of Scharschmidt *et al.* (1979). Cytochrome oxidase and NADPH:cytochrome *c* reductase, markers for mitochondrial membranes and

Fig. 1. Glucose uptake by basolateral-membrane vesicles

Vesicles were prepared in 300 mM-mannitol/20 mM-Hepes/Tris buffer, pH 7.4. Reaction was started by the addition of 20 μ l of membrane vesicles to a medium containing (final concns.) (■) 100 mM-NaCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer, pH 7.4, 0.1 mM-D-glucose and tracer [³H]glucose, or (●) 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, 0.1 mM-D-glucose and tracer [³H]glucose. Reaction was stopped at 20 s, 2, 5 and 60 min. Values are means $\pm$ S.E.M. for three separate experiments.

endoplasmic reticulum respectively, were measured as described by Beaufay *et al.* (1974).

RESULTS

Purity of membrane vesicles

Marker-enzyme studies. The specific activity of Na⁺ + K⁺-ATPase, a marker for basolateral membranes, was enriched 9 $\pm$ 2-fold as compared with mucosal homogenate; leucine aminopeptidase and disaccharidases (brush-border-membrane markers) were impoverished 0.8 $\pm$ 0.2-fold. Cytochrome *c* oxidase (mitochondrial membrane marker) was impoverished 0.6 $\pm$ 0.1-fold and NADPH:cytochrome *c* reductase (endoplasmic-reticulum marker) 0.7 $\pm$ 0.1-fold.

Orientation of the basolateral membrane. The orientation of the basolateral-membrane vesicles was determined by the latency of the Na⁺ + K⁺-ATPase activity. The activity in basolateral membranes was measured before and after treatment with 0.06% deoxycholate/1.0 mM-EDTA. Inside-out vesicles represented 59% of the total vesicles, as evidenced by the differences in non-inhibitable ATPase activity (Table 1).

D-Glucose uptake by basolateral-membrane vesicles

Fig. 1 depicts D-glucose uptake in the presence and absence of a Na⁺ gradient: no 'overshoot' was noted. Moreover, there was no difference in uptake in the presence of Na⁺ or K⁺ gradients. Intravesicular volume, calculated from D-glucose equilibrium values, was 2.1 $\pm$ 0.2 μ l/mg of protein. Similar intravesicular volumes were obtained from [¹⁴C]mannitol uptake studies at equilibrium (results not shown).

Ca²⁺ uptake by basolateral-membrane vesicles

Fig. 2 depicts Ca²⁺ uptake in the presence and absence of ATP; that in the presence of ATP was severalfold greater than in the absence of ATP. Moreover, an 'overshoot' was seen in the presence of ATP, compared with no-ATP conditions.

Table 1. Effect of treatment with deoxycholate/EDTA on Mg²⁺-ATPase and Na⁺ + K⁺-ATPase in basolateral membranes

Values are means $\pm$ S.E.M. for three different determinations. ATPase activity is expressed as nmol of P_i/min per mg of protein. Basolateral-membrane vesicles were treated with a mixture of deoxycholate (DOC) and EDTA (final concns. 0.06 mM and 1 mM respectively) for 30 min at room temperature. Immediately after this treatment, the samples were analysed for enzyme activities. Ion concentrations: Mg²⁺, 5 mM; Na⁺, 100 mM; K⁺, 20 mM; ouabain 1 mM.

Incubation medium	ATPase activity	
	-DOC/ EDTA	+DOC/ EDTA
Mg ²⁺ + Na ⁺ + K ⁺ + ouabain	180 $\pm$ 30	210 $\pm$ 10
Mg ²⁺ + Na ⁺ + K ⁺	230 $\pm$ 60	330 $\pm$ 60
Na ⁺ + K ⁺ -ATPase	50	120
	(41%)	(100%)

Fig. 2. Ca^{2+} uptake by basolateral-membrane vesicles

Vesicles were prepared in 200 mM-mannitol/100 mM-KCl/20 mM-Hepes/Tris buffer, pH 7.4. Reaction was started by the addition of 20 μl of membrane vesicles to a medium with a similar composition except for the addition of (■) 5 mM-MgATP, 0.1 mM- CaCl_2 and tracer ^{45}Ca , or (□) no ATP, 0.1 mM- CaCl_2 and tracer ^{45}Ca . Reaction was stopped at 30 s, 1, 5, 10, 30, 60 and 120 min. Values are means $\pm$ S.E.M. for three separate experiments.

Fig. 3. Effect of osmolarity on phosphate uptake

Vesicles were prepared in 200 mM-mannitol/100 mM-KCl/20 mM-Hepes/Tris buffer, pH 7.4. Reaction was started by the addition of 20 μl of membrane vesicles to a different medium containing 100 mM-KCl and increasing amounts of mannitol to give osmolarities between 200 and 500 mosM, 0.1 mM-phosphate and ^{32}P . Reaction was stopped at 90 min. Values are means $\pm$ S.E.M. for three experiments.

Osmolarity

To determine whether phosphate uptake is into an osmotically active space, or represents mere binding, vesicles were incubated in solutions of various osmolarities, and phosphate uptake was determined at equilibrium (90 min); Fig. 3 depicts the results. At equilibrium, all uptake represented transport into the intravesicular space.

Phosphate uptake with time

Uptake of phosphate was determined as a function of time and cationic gradient. Basolateral-membrane vesicles were prepared in mannitol/KCl buffer and incubated

Fig. 4. Time course of phosphate uptake

Vesicles were prepared in 100 mM-KCl/100 mM-mannitol/20 mM-Hepes/Tris, pH 7.4. Reaction was started by the addition of 20 μl of vesicles to a medium containing (final concns.) either (□) 100 mM-NaCl, 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, 0.1 mM-phosphate and tracer ^{32}P , or (●) 100 mM-choline chloride, 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, 0.1 mM-phosphate and tracer ^{32}P . Reaction was stopped at 30 s, 1, 5, 15, 30, 60 and 90 min. Values are means $\pm$ S.E.M. for three separate experiments.

in a Na^+ - or choline-containing buffer, so that a 100 mM cationic gradient was directed from the extravesicular to the intravesicular space. Fig. 4 depicts phosphate uptake with time in the presence of Na^+ and choline gradients. In the presence of a Na^+ gradient, phosphate uptake showed an 'overshoot' over equilibrium values at 60 min. In the presence of a choline gradient, uptake did not exhibit an 'overshoot'. Intravesicular space, calculated from equilibrium values, was $2 \pm 0.1 \mu\text{l}/\text{mg}$ of protein, which was similar to values obtained with D-glucose and mannitol.

Effect of electrical potential

The effect of an imposed electrochemical membrane potential was studied by using two methods: (1) valinomycin-induced K^+ diffusion potential and (2) anion substitution. In the first experiments, membrane potential was induced by preincubating the vesicles with 100 mM-KCl, and the reaction was started by addition of the vesicles to media containing 100 mM-NaCl and valinomycin. Uptake values were compared with those obtained under voltage-clamp conditions (i.e. incubation of 100 mM-KCl-preloaded vesicles in 100 mM-KCl and valinomycin; K^+ outside = K^+ inside). Because valinomycin mediates the electrogenic movement of K^+ down its concentration gradient, a negative membrane potential is generated. Table 2 depicts that at 10 s and 1 min there was no significant difference with negative membrane potential, indicating an electroneutral process of uptake. To resolve the question of membrane potential further, the effect of Na^+ salts with different anion permeabilities was tested on phosphate uptake. NaNO_3 represents a highly permeable anion compared with Na_2SO_4 , and NaCl permeability is intermediate. Because NaNO_3 enters the vesicles faster, a negative membrane potential is generated earlier than with Na_2SO_4 . Table 3 depicts phosphate uptake at 10 s and

Table 2. Effect of negative membrane potential induced by valinomycin on phosphate uptake

Vesicles were prepared in 100 mM-KCl, 100 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4. Reaction was started by the addition of 20 μ l of vesicles to a medium containing either 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, and valinomycin (10 μ g/mg of protein) or 100 mM-KCl, 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, and valinomycin (10 μ g/mg of protein) (voltage-clamp condition). Reaction was stopped at 10 s and 1 min. Values are means $\pm$ S.E.M. for three separate experiments.

Time	Phosphate uptake (pmol/mg of protein)	
	Negative potential	Voltage clamp
10 s	22.2 $\pm$ 3.7	2.3 $\pm$ 3.5
1 min	77.7 $\pm$ 10.4	71.3 $\pm$ 4.3

Table 3. Effect of negative membrane potential induced by anions on phosphate uptake

Vesicles were prepared in 100 mM-KCl, 100 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4. Reaction was started by the addition of 20 μ l of vesicles to a medium containing 100 mM-NaSCN, 100 mM-NaCl or 50 mM-Na₂SO₄. Incubation media also contained 20 mM-Hepes/Tris buffer, pH 7.4, 0.1 mM-phosphate and ³²P. Reaction was stopped at 10 s and 1 min. Values are means $\pm$ S.E.M. for three separate experiments.

Time	Phosphate uptake (pmol/mg of protein)		
	NaSCN	NaCl	Na ₂ SO ₄
10 s	52.2 $\pm$ 10.2	61.1 $\pm$ 5.2	50 $\pm$ 12.2
1 min	73.3 $\pm$ 11.1	91.0 $\pm$ 9.5	80 $\pm$ 12.2

Fig. 5. Initial rate of phosphate uptake

Vesicles were prepared in 100 mM-mannitol/100 mM-KCl/20 mM-Hepes/Tris buffer, pH 7.4. Reaction was started by the addition of 20 μ l of vesicles to a medium containing 100 mM-NaCl, 100 mM-mannitol, 20 mM-Hepes/Tris, pH 7.4, 0.1 mM-phosphate and ³²P. Reaction was stopped at 3, 4, 10 and 15 s. Values are means $\pm$ S.E.M. for three experiments.

Fig. 6. Kinetics of phosphate uptake

Vesicles were prepared in 100 mM-mannitol/100 mM-KCl/20 mM-Hepes/Tris buffer, pH 7.4. Reaction was started by the addition of 20 μ l of vesicles to a medium containing either (□) 100 mM-NaCl, 100 mM-mannitol and 20 mM-Hepes/Tris or (●) 100 mM-choline chloride, 100 mM-mannitol and 20 mM-Hepes/Tris. Phosphate concentration in each incubation media ranged between 0.01 and 1 mM, and tracer ³²P was present. Reaction was stopped at 10 s. The active component of uptake (○) was calculated from the difference of phosphate uptake under Na⁺-gradient conditions minus uptake under choline-gradient conditions. *K_m* and *V_{max}* of the active component were derived from a computerized model of Michaelis-Menten kinetics, and were 14.0 $\pm$ 3.9 μ M and 51.1 $\pm$ 4.2 pmol/10 s per mg of protein.

1 min in the presence of different Na⁺ salts; there was no significant difference in uptake values, indicating again an electroneutral process.

Initial rate of phosphate uptake

To determine the kinetics of phosphate uptake, it was essential to determine the linearity of uptake with time. Fig. 5 depicts phosphate uptake in the presence of a Na⁺ gradient up to 15 s, which was linear. Therefore, kinetic studies were determined at 10 s.

Kinetics of phosphate uptake

Fig. 6 depicts phosphate uptake in the presence of Na⁺ and choline gradients at 10 s. The incubation media contained various concentrations of phosphate (0.01–0.1 mM). The kinetic data were analysed by using uptake values with the Na⁺ gradient minus uptake with the choline gradient. *V_{max}* was 51.1 $\pm$ 4.2 pmol/10 s per mg of protein and *K_m* was 14 $\pm$ 3.9 μ M.

Tracer exchange studies

To provide further evidence for the presence of a phosphate carrier, tracer-exchange studies were conducted. Vesicles were preincubated in 100 mM-NaCl/100 mM-mannitol/0.1 mM-phosphate/20 mM-Hepes/Tris buffer, or in 100 mM-KCl/100 mM-mannitol/0.1 mM-phosphate/20 mM-Hepes/Tris buffer, pH 7.4. The reaction was started by diluting the vesicles in a similar medium except for the addition of ³²P. Table 4 shows that transport in the presence of equilibrated Na⁺ was greater than in the presence of K⁺-equilibrated conditions.

Table 4. Trace exchange of phosphate by basolateral-membrane vesicles

Values are means $\pm$ S.E.M. for three different experiments. In the Na⁺-equilibrated conditions, vesicles were preincubated in 100 mM-NaCl/100 mM-mannitol/0.1 mM-phosphate/20 mM-Hepes/Tris buffer, pH 7.4. Reaction was started by incubating the vesicles in a similar medium except for the addition of tracer ³²P and gramicidin (10 mg/mg of protein). In the K⁺-equilibrated condition a similar procedure was used, except for substitution of K⁺ for Na⁺. Samples were taken at 10 s and 1 and 60 min.

Experimental condition	Concn. of phosphate (mM) (in/out)	Tracer phosphate uptake (c.p.m.)		
		10 s	1 min	60 min
Na ⁺ equilibration	0.1/0.1	747 $\pm$ 120	1680 $\pm$ 214	2021 $\pm$ 340
K ⁺ equilibration	0.1/0.1	308 $\pm$ 26	431 $\pm$ 11.5	213 $\pm$ 320

Table 5. Trans-stimulation of phosphate transport (counter-transport) by basolateral membranes

Values are means $\pm$ S.E.M. for three different experiments. Vesicles were preincubated with or without 0.1 mM-phosphate for 1 h at 25 °C. In the Na⁺-gradient conditions, a 100 mM-Na⁺ gradient was directed to the inside. In the K⁺-gradient conditions, a 100 mM-K⁺ gradient was directed to the inside. ³²P tracer was added to the incubation media in both the Na⁺ and K⁺ gradient condition. Samples were taken at 10 s and 1 and 60 min. * Significant difference from corresponding mean values under K⁺-gradient condition ($P < 0.01$); † significant differences from corresponding mean value under no-trans-stimulation conditions ($P < 0.01$).

Experimental condition	Concn. of phosphate (mM) (in/out)	Tracer phosphate uptake (c.p.m.)		
		10 s	1 min	60 min
Na ⁺ gradient out > in	0.1/tracer ³² P	2143 $\pm$ 240*†	2810 $\pm$ 270*†	2810 $\pm$ 280
	0/tracer ³² P	951 $\pm$ 151	1397 $\pm$ 120	3445 $\pm$ 270
K ⁺ gradient out > in	0.1/tracer ³² P	877 $\pm$ 20	710 $\pm$ 210	2786 $\pm$ 120
	0/tracer ³² P	844 $\pm$ 50	1186 $\pm$ 200	2700 $\pm$ 240

Trans-stimulation studies (counter-transport)

In order to confirm the presence of a phosphate carrier, counter-transport studies were conducted. Vesicles were incubated in media containing 300 mM-mannitol and 20 mM-Hepes/Tris, with or without 0.1 mM-phosphate. Reaction was started by the addition of vesicles to a medium containing either 100 mM-NaCl, 100 mM-mannitol, 20 mM-Hepes/Tris buffer and tracer ³²P, or 100 mM-mannitol, 100 mM-KCl, 20 mM-Hepes/Tris buffer and ³²P tracer. As shown in Table 5, in the presence of phosphate on the *trans* side of the membrane, and an inwardly directed Na⁺ gradient, a significant uptake of phosphate occurred compared with no-trans-stimulation conditions. In the presence of K⁺, there was no stimulation by the presence of phosphate on the *trans*-side of the membrane.

Stoichiometry of phosphate transport

To determine the number of Na⁺ ions transported with phosphate, the 'static head' method was used as described by Turner & Moran (1982), who published the rationale for this method. Briefly, the coupling ratio of Na⁺ for the transporter was determined by comparing the efflux of phosphate in the absence of Na⁺ with that in the presence of intravesicular Na⁺ and an inwardly directed external Na⁺ gradient. The formula depicted below fulfils the conditions in which no net efflux of coupled Na⁺ and phosphate exists:

$$\ln ([P]_i/[P]_o) = n \ln ([Na]_o/[Na]_i)$$

where [P]_i and [P]_o are the internal and external phosphate concentration and [Na]_i and [Na]_o are the respective concentration of sodium. Fig. 7 depicts the

stoichiometry of Na⁺ for Na⁺-phosphate-dependent uptake; it appears that, at pH 7.4, 2 Na⁺ ions are coupled to HPO₄²⁻.

Effect of positive membrane potential on phosphate uptake by basolateral membranes

To investigate whether the Na⁺ effect on transport is due to generating a positive membrane potential or to the presence of Na⁺-P_i co-transporter, we studied phosphate uptake in the presence of inside-positive potential. Table 6 depicts phosphate uptake at 10 s, and 1, 5 and 60 min. Inside-positive potential resulted in a slight decrease in phosphate uptake, compared with voltage-clamp conditions.

DISCUSSION

The current studies were designed to characterize phosphate transport across the basolateral membranes of the rat enterocyte. We have isolated purified intestinal basolateral membranes by using a Percoll density gradient; morphological, biochemical and functional studies demonstrated lack of contamination by brush-border membrane vesicles. The studies demonstrate the presence of a Na⁺-P_i co-transporter in the basolateral membrane. The presence of this transporter was verified by *trans*-stimulation and tracer-exchange studies. The process was electroneutral and exhibited a 2 Na⁺:1 phosphate stoichiometry. The kinetics of this transport system showed a V_{max} of 51.1 $\pm$ 4.2 pmol/10 s per mg of protein and a K_m of 14 $\pm$ 3.9 μ M. These values are similar to those obtained with dog kidney basolateral-membrane vesicles (Schwab *et al.*, 1984). These kinetic parameters are lower than corresponding kinetics of

Fig. 7. Stoichiometry of phosphate transport

Vesicles were prepared in 40 mM-KCl/20 mM-NaCl/20 mM-Hepes/Tris/180 mM-choline chloride/0.15 mM-phosphate containing ^{32}P . The control flux (□) contained 20 mM-choline chloride instead of 20 mM-NaCl. Reaction was started by addition of vesicles to a medium containing 20 mM-Hepes/Tris, 40 mM-KCl and 57 mM-NaCl (●; $n = 1.6$), 47 mM-NaCl (○; $n = 2$) or 40 mM-NaCl (■; $n = 2.5$) and sufficient choline chloride to equal the internal osmolarity; n is the number of Na^+ ions derived from the equation given in the text. Efflux from vesicles containing 20 mM-NaCl represents total flux in the presence of an external Na^+ gradient. Efflux is expressed as a percentage of the total at zero time. Values are means $\pm$ S.E.M. for three separate experiments.

Table 6. Effect of inside-positive membrane potential on phosphate uptake by basolateral membranes

Values are means $\pm$ S.E.M. for two experiments. Inside-positive was generated by preincubating vesicles with 300 mM-mannitol and 20 mM-Hepes/Tris buffer, pH 7.4. Incubation media contained 100 mM-KCl, 20 mM-Hepes/Tris and valinomycin (10 mg/mg of protein). In the voltage-clamp conditions, vesicles were preincubated with 100 mM-KCl and 20 mM-Hepes/Tris buffer. Incubation medium was similar, except for the addition of valinomycin (10 μg /mg of protein). Samples were taken at 10 s and 1, 5 and 60 min.

Time	Phosphate uptake (pmol/mg of protein)	
	Inside positive	Voltage clamp
10 s	38 $\pm$ 8	42 $\pm$ 10
1 min	39 $\pm$ 7	53 $\pm$ 10
5 min	49 $\pm$ 5	55 $\pm$ 8
60 min	165 $\pm$ 15	178 $\pm$ 12

phosphate uptake by brush-border membranes (K_m 70 μM , and V_{max} 1766 pmol/10 s per mg of protein) (Borowitz & Ghishan, 1985). Therefore, compared with Na^+ -gradient-dependent P_i transport in brush-border-membrane vesicles, transport in basolateral-membrane vesicles appears to have higher affinity and lower

capacity. The critical question arises whether this transporter represents merely contamination by brush-border membranes or whether it is truly located at the basolateral membrane. Hoffman *et al.* (1976) studied phosphate transport in basolateral-membrane vesicles isolated from rat kidney. Their findings demonstrated increased phosphate uptake in basolateral vesicles in the presence of Na^+ gradient compared with that measured in the presence of a K^+ gradient. They attributed this effect of Na^+ on phosphate transport to contamination with brush-border membranes. However, Schwab *et al.* (1984) in a detailed study demonstrated the presence of a Na^+-P_i transporter in kidney basolateral-membrane vesicles. This transporter has a different kinetic system. Therefore, based on their findings, those authors suggested that the transporter cannot be due to contamination with brush-border membrane. Similarly, our studies demonstrate a different kinetic system for phosphate transport in the brush-border and basolateral membrane. Previous studies in our laboratory (Borowitz & Ghishan, 1985) showed that the V_{max} for phosphate transport in rat brush-border-membrane vesicles was 1766 pmol/10 s per mg of protein, which is 35 times values obtained for brush-border membranes. Similarly the K_m value for phosphate uptake by brush-border membranes was 5 times values obtained with basolateral membranes. Therefore we believe that a phosphate transporter is present in the basolateral membrane. This transporter does not appear to be due to contamination with brush-border membranes, as shown by differences in the kinetics of the two transporters, as well as by the lack of 'overshoot' phenomena with D-glucose, and the ATP-dependency of Ca^{2+} uptake by our basolateral membrane preparation.

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