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SODIUM-DEPENDENT PHOSPHATE TRANSPORT BY APICAL MEMBRANE VESICLES FROM A CULTURED RENAL EPITHELIAL CELL LINE (LLC-PK_1)

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Apical membrane vesicles were prepared from confluent monolayers of LLC-PK_1 cells grown upon microcarrier beads. The final membrane preparation, obtained by a modified divalent cation precipitation technique, was enriched in alkaline phosphatase, leucine aminopeptidase and trehalase (8-fold compared to the initial homogenate). Analysis of phosphate uptake into the vesicles identified a specific sodium-dependent pathway. Lithium and other cations were unable to replace sodium. At 100 mmol/l sodium and pH 7.4, an apparent K_m for phosphate of 99 ± 19 μmol/l and an apparent K_i for arsenate of 1.9 mmol/l were found. Analysis of the sodium activation of phosphate uptake gave an apparent K_m for sodium of 32 ± 12 mmol/l and suggested the involvement of two sodium ions in the transport mechanism. Sodium modified the apparent K_m of the transport system for phosphate. The rate of sodium-dependent phosphate uptake was higher at pH 6.4 than at pH 7.4. At both pH values, an inside negative membrane potential (potassium gradient plus valinomycin) had no stimulatory effect on the rate of the sodium-dependent component of phosphate uptake. It is concluded that the apical membrane of LLC-PK_1 cells contains a sodium-phosphate cotransport system with a stoichiometry of 2 sodium ions: 1 phosphate anion.

Introduction

In the last ten years, epithelial cell lines derived from various transporting epithelia have gained wide acceptance as useful 'models' for the study of the mechanics of transepithelial ion and solute fluxes [1,2]. Under standard culture conditions these cell lines express many of the morphological, biochemical and functional parameters normally associated with their parent tissue. In this respect, a renal epithelial cell line: LLC-PK_1 [3] derived from pig kidney has been extensively employed in the study of transport events normally associated with renal proximal tubular epithelium, such as sodium-dependent hexose transport [4,5] and amino acid transport [6,7].

In a recent publication we described how, in addition to sugar and amino acid transport, LLC-PK_1 cells retain the ability to transport inorganic phosphate [8]. Phosphate uptake into subconfluent cell monolayers of LLC-PK_1 cells was mediated by a Na^+-dependent, substrate-saturable process which was sensitive to inhibition by arsenate.

To further characterise the phosphate transporter of LLC-PK_1 we decided to exploit the advantages inherent in the use of isolated membrane vesicles to study the kinetics of phosphate transport [9,10]. This present work therefore, describes a method for the preparation of well defined apical membrane vesicles from monolayers of LLC-PK_1 cells grown upon microcarrier beads. A microcarrier culture system provided the most

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid.

efficient way to culture the large number of cells needed for membrane isolation. The major result of this paper is the identification and characterisation of a sodium-dependent phosphate transport system upon the apical membrane of LLC-PK_1 which shares many properties in common with the phosphate transport system identified in renal and intestinal brush-border membranes [11].

Materials and Methods

Cell culture. LLC-PK_1 pig kidney epithelial cells [3] were originally obtained from Flow Laboratories (Irvine, Scotland) at 145 serial passages. Stocks of these cells, from which all subsequent passages were made, are held in a liquid nitrogen safe in this laboratory.

Routinely, the cell-line was maintained in serial culture in 120 cm^2 (growth area) Roux flasks, at 37°C in a 95% air/5% CO_2 atmosphere. The growth media used throughout this study was Dulbecco's modification of Eagle's minimum essential media supplemented with 5% (v/v) foetal calf serum, 5% (v/v) donor horse serum, 1% non essential amino acids and penicillin (100 I.U./ml)/streptomycin (100 μg/ml).

For membrane isolation, LLC-PK_1 cells were grown as monolayers upon a microcarrier bead substrate [12]. Cytodex 1™ microcarrier beads were hydrated and sterilised according to the manufacturer's (Pharmacia, Uppsala, Sweden) instructions and suspended in growth media by gentle stirring using Techne spinner flasks and microcarrier spinner unit (Techne, Cambridge, U.K.). Cultures were initiated by seeding the microcarriers with trypsinised LLC-PK_1 cells at a density of $1 \cdot 10^8$ cells per gram of beads in 100 cm^3 growth media (the usual culture size was 2 g of beads). After an initial 30 min period of static attachment, the cultures were stirred at 15 rpm for 3 h. The cultures were then diluted to 500 cm^3 with fresh media and stirred at 25–30 rpm for the remainder of the culture period. On day 3 of culture 50% of the media was replaced. Cultures were used for experimental purposes when they attained confluency (4–6 days in culture).

Membrane isolation. Confluent cell monolayers on microcarrier beads were separated from the growth media by a short centrifugation step (1000 $\times g$, 2 min, SS-34 rotor). The cells and cell fragments were then separated from the microcarrier substrate by resuspending the pellet in a hypotonic media (30 mmol/l mannitol, 20 mmol/l Hepes-Tris) at pH 8 and 4°C, and vortex mixing for 1 min. This manouver causes a rapid detachment of the cell monolayer from the bead substrate [13]. To ensure maximum recovery of cells a mild sonication step (80 kHz, 0.5 A, 60 s (Type G-112, Lab. Supplies Co., Hicksville, NY, U.S.A.)) was also included. The isolated cells were separated from the microcarrier matrix by filtration through a 100 μm nylon mesh. The beads were then washed with 60 cm^3 of buffer to maximize the recovery of cells. The filtrate was centrifuged at 36000 $\times g$ for 15 min in a Sorvall SS-34 rotor. The pellets (P_1) were then resuspended in a small volume of buffer (300 mmol/l mannitol, 20 mmol/l Hepes-Tris, pH 7.4) and homogenised using a Polytron homogeniser (Type PT-10, setting 4, 4 min). $MgCl_2$ was added to the homogenate to a final concentration of 10 mmol/l. After 30 min at 4°C the homogenate was centrifuged for 15 min at 2400 $\times g$ in a Sorvall SS-34 rotor. The pellet (P2) was kept for enzyme analysis. The supernatant (S2) was centrifuged for 45 min at 30000 $\times g$ in a Sorvall SS-34 rotor. The pellet (P3) was resuspended in the appropriate uptake buffer (usually 300 mmol/l mannitol, 20 mmol/l Hepes-Tris, pH 7.4) by repeated syringing through a fine needle.

Assays of enzyme activities and protein. Enzyme activities were assayed, if possible, on the same day as the membranes were isolated. Enzyme activities were determined, semi-automatically, using either a LKB reaction rate analyser (model 8600) or the kinetic analysis programme of a Bausch and Lomb spectrophotometer (model 2000). The activities of alkaline phosphatase (EC 3.1.3.1) and ouabain sensitive ($Na^+ + K^+$)-ATPase (EC 3.6.1.3) were assayed as described by Berner and Kinne [14]. Leucine aminopeptidase (EC 3.4.11.2) was determined as described by Haase et al. [15]. Trehalase activity (EC 3.2.1.28) was determined in a similar manner to that described for maltase by Evers et al. [16] and by Rohn et al. [17], except that trehalose was used as the substrate. The activities of KCN-resistant NADH-oxidoreductase (EC 1.6.99.2) and of succinate-cytochrome *c*-oxidoreductase (EC 1.3.99.1) were assayed according to

Sottocasa et al. [18] and Fleischer and Fleischer [19], respectively. All enzyme activities were measured at 37°C. Protein was determined by the method of Bradford [20] using Bio-Rad reagents.

Transport studies. Uptake of labelled inorganic phosphate into isolated membrane vesicles from LLC-PK_1 cells was measured with a rapid filtration technique as previously described [9,21]. The compositions of the internal and external buffers and the substrate concentration for each uptake condition are described in the appropriate figure legend.

Materials. All cell culture reagents were obtained from Flow Laboratories, Baar, Switzerland. All other chemicals were of the highest available purity. Radio-isotopes were obtained from New England Nuclear Corporation, Boston, MA, U.S.A.

Results

Enzymatic characterisation of membrane preparation

Tables IA and IB summarise the recoveries, specific activities and enrichment factors of several marker enzymes obtained during the membrane fractionation of LLC-PK_1 cells. Alkaline phosphatase, leucine aminopeptidase and trehalase were used as apical membrane markers [22]. ($Na^+ + K^+$)-ATPase for the basal-lateral membrane [10] and KCN-resistant NADH oxidoreductase and succinate-cytochrome *c*-oxidoreductase for the endoplasmic reticulum and mitochondria, respectively.

Table IB shows that the specific activities of brush-border marker enzymes: alkaline phos-

TABLE IA

RECOVERIES OF MARKER ENZYMES OBTAINED DURING THE ISOLATION OF BRUSH-BORDER MEMBRANE VESICLES FROM LLC-PK_1 CELLS

Enzyme recovery is expressed as a percentage of the enzyme activity in the initial homogenate. The results are the mean ± S.D. of *n* individual experiments. n.d., not detected.

Enzyme	Enzyme recovery (%)			
Membrane fraction	P_2	Brush border (P_3 fraction)	Sup_2	Total recovery
Alkaline phosphatase ($n = 7$)	40.2 ± 8.2	38.1 ± 7.5	n.d.	78.3 ± 9.5
Leucine aminopeptidase ($n = 7$)	31.0 ± 5.2	31.2 ± 6.9	n.d.	62.1 ± 9.2
Trehalase ($n = 3$)	35.0 ± 6.0	40.0 ± 5.0	n.d.	75.0 ± 7.2
($Na^+ + K^+$)-ATPase ($n = 5$)	48.6 ± 14.8	5.4 ± 3.0	n.d.	54.0 ± 13.9
KCN-resistant NADH oxidoreductase ($n = 5$)	60.5 ± 8.8	3.0 ± 1.1	n.d.	60.2 ± 7.5
Succinate-cytochrome *c* oxidoreductase ($n = 5$)	66.8 ± 10.6	5.0 ± 3.3	n.d.	71.3 ± 11.5

TABLE IB

SPECIFIC ACTIVITIES AND ENRICHMENT FACTORS OF MARKER ENZYMES IN THE BRUSH-BORDER MEMBRANE FRACTION

All enzyme activities are given in μmol/min per mg protein except for trehalase which is expressed as μmol/h per mg protein.

Enzyme	Specific activity		
	Homogenate (P_3 fraction)	Brush border factor	Enrichment
Alkaline phosphatase	318.2 ± 38.1	2086.7 ± 24.8	7.2 ± 0.5
Leucine aminopeptidase	16.4 ± 2.9	93.2 ± 11.2	6.1 ± 0.6
Trehalase	0.41 ± 0.05	3.19 ± 0.5	7.8 ± 0.1
($Na^+ + K^+$)-ATPase	13.8 ± 3.3	7.4 ± 1.9	0.7 ± 0.2
KCN-resistant NADH oxidoreductase	202.2 ± 49.7	80.4 ± 14.1	0.6 ± 0.1
Succinate-cytochrome *c* oxidoreductase	17.7 ± 4.8	7.8 ± 2.9	0.4 ± 0.1

phatase, leucine aminopeptidase and trehalase are enriched 7- to 8-fold in the P3 fraction compared to the initial homogenate. The P3 fraction contains roughly 30–40% of the total activities of these enzymes (Table IA). In contrast to the enrichment of brush-border enzymes, the P3 fraction was significantly depleted of $(Na^+ + K^+)$-ATPase, NADH oxidoreductase and succinate-cytochrome *c*-oxidoreductase activities (Table IB) compared to the homogenate. Similarly the recoveries of these enzymes in the final pellet was at most 5% of the total activity. These results suggest that the isolation procedure produces relatively pure brush border membranes.

Time-course and Na^+ stimulation of phosphate uptake

The time-course of phosphate uptake into apical membrane vesicles of LLC-PK_1 cells is shown in Fig. 1. Phosphate uptake into LLC-PK_1 apical membrane vesicles was markedly stimulated in the presence of an initial 100 mmol/l NaCl gradient compared to uptake in the presence of a 100 mmol/l KCl gradient. Uptake in the presence of sodium was linear over the first minute of measurement and reached the equilibrium value within the first 10 min. A transient 'overshoot' of intravesicular phosphate above its equilibrium value was, however, not observed.

Cation dependence of phosphate uptake

Fig. 2 shows the effect of stepwise replacement of Na^+ by choline upon the initial uptake velocity of 100 μmol/l phosphate at pH 7.4. An increased rate of phosphate uptake was observed as the extravesicular Na^+ concentration was raised. At physiological sodium concentrations (140 mmol/l) the transport system was saturated. At low extravesicular Na^+ concentrations the curve tended to be sigmoidal, which can indicate that more than one sodium ion interacts with the phosphate transport system. Kinetic analysis of the data supported this view, a double reciprocal plot of $1/v$ against $1/[Na^+]^2$ gave a straight line ($r^2 = 0.964$), similarly a Hill plot of the same data gave a Hill coefficient of 1.6 ± 0.2 ($r = 0.90$). These data are consistent with a model in which the stoichiometry between Na^+ and phosphate is 2:1 [23]. The apparent K_m of the transport system for Na^+, at 100 μmol/l phosphate, pH 7.4, was calculated to be 31.6 ± 12 mmol/l Na^+. The requirement of the transport system for Na^+ was specific, 100 mmol/l gradients of Li^+, K^+, Cs^+ or choline were ineffective in stimulating phosphate uptake.

Interaction of phosphate with the transport system

Fig. 3a shows the effect of changing the extravesicular phosphate concentration upon the ini-

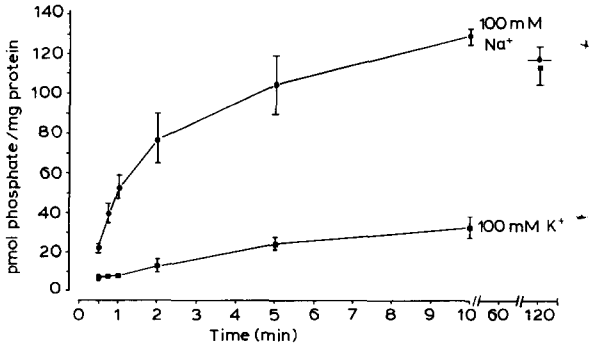

Fig. 1. Time dependence of phosphate uptake by LLC-PK_1 apical membrane vesicles. The vesicles contained 300 mmol/l mannitol and 20 mmol/l Hepes-Tris buffer, pH 7.4. Phosphate uptake was measured in an incubation media containing in addition 0.1 mmol/l phosphate ($^{32}P = 0.5$ μCi/20 μl) and 100 mmol/l NaCl (●———●) or KCL (■———■). The results are expressed as the mean ± S.D. of six determinations. The protein concentration per time point was 0.05 mg.

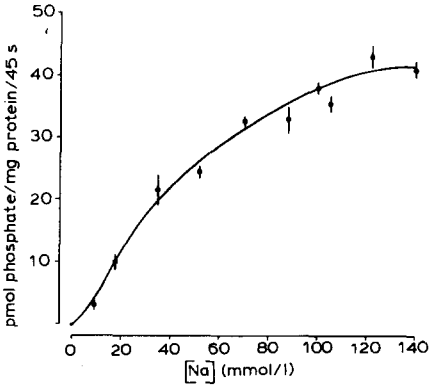

Fig. 2. Influence of sodium upon phosphate uptake by LLC-PK_1 apical membrane vesicles. The vesicles contained 300 mmol/l mannitol and 20 mmol/l Hepes-Tris, pH 7.4. The incubation media contained in addition 0.1 mmol/l KH_2PO_4 and various concentrations of NaCl (NaCl replaced isoosmotically by choline chloride). Uptake was measured over a 45 s period and is corrected for uptake in the absence of Na^+. Each value represents the mean ± S.D. of three determinations.

tial uptake velocity of phosphate into apical membrane vesicles. Two lines are shown: uptake in the presence of 100 mmol/l K^+, which changed very little over the range of phosphate concentrations tested, and the net sodium-dependent component of phosphate uptake. The sodium-dependent component of uptake was markedly dependent upon extravesicular phosphate concentration and was a saturable process. Kinetic analysis of the sodium-dependent component of phosphate uptake (Lineweaver-Burk plot) gave an apparent K_m of 99.2 ± 19 μmol/l ($r = 0.952$) and an apparent maximum velocity of 75.5 ± 16 pmol/mg per 45 s.

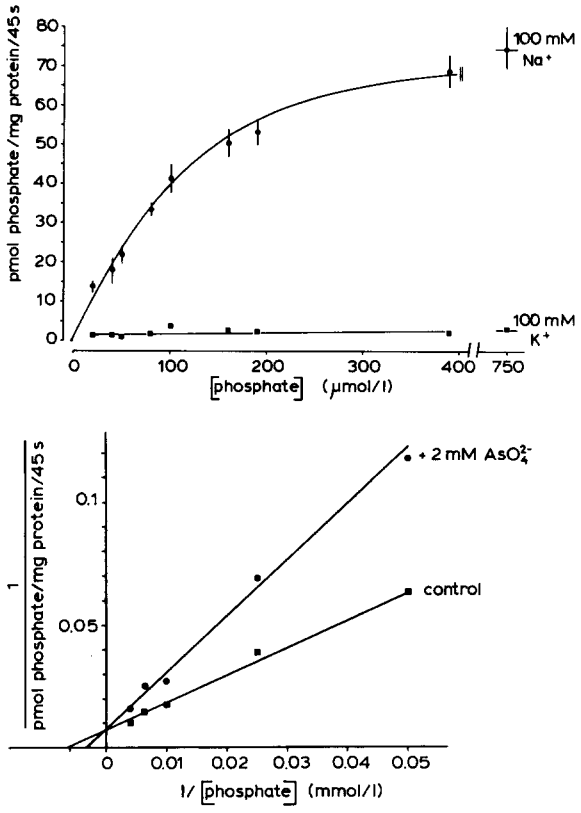

Fig. 3. (a) Influence of phosphate upon phosphate uptake by LLC-PK_1 apical membrane vesicles. Vesicles contained 300 mmol/l mannitol and 20 mmol/l Hepes-Tris, pH 7.3. The incubation media contained in addition 100 mmol/l NaCl (●———●) or 100 mmol/l KCl (■———■) and various concentrations of KH_2PO_4 (^{32}P-labelled). Uptake was measured over a 45 s uptake period. The data represents the mean ± S.D. of four determinations. (b) Lineweaver-Burk plot of the inhibition of the Na^+-dependent component of phosphate uptake by arsenate into LLC-PK_1 apical membrane vesicles. Uptake conditions were the same as in Fig. 3a, except that in addition uptake was also measured in the presence of 2 mmol/l arsenate. The data (corrected for uptake in a Na^+-free media) shows that in the presence of arsenate the apparent K_m for phosphate is increased from 142 ± 25 μmol/l (control) to 333 ± μmol/l. Arsenate has no effect upon the apparent V_{max} of 125 pmol/mg per 45 s. Each data point is the mean of three determinations.

As in a number of other phosphate transport systems [24,25] arsenate was found to inhibit a greater proportion of the Na^+-dependent phosphate uptake in LLC-PK_1 apical membrane vesicles with a maximal inhibition of 70% at 5 mmol/l arsenate. Analysis of the effect of 2 mmol/l arsenate upon the sodium-dependent component of phosphate uptake at different extravesicular phosphate concentrations (Fig. 3b) shows that arsenate acts as a competitive inhibitor of phosphate uptake, changing the apparent affinity of the system for phosphate from 142 ± 25 μmol/l to 333 ± 30 μmol/l plus arsenate. Arsenate had no apparent effect upon the V_{max} of the transport system. When the data presented in Fig. 3b was replotted as a Dixon plot [26] a K_i for the ability of arsenate to inhibit Na^+-dependent phosphate uptake of 1.9 mmol/l was obtained.

Analysis of phosphate uptake at two different Na^+ concentrations (40 and 100 mmol/l) clearly demonstrates (Fig. 4) that Na^+ stimulates phosphate uptake by modifying the affinity of the transport system for phosphate. The apparent K_m for phosphate changes from 89.2 μmol/l at 100 mmol/l Na^+ to 312.5 μmol/l when extravesicular Na^+ is reduced to 40 mmol/l. In contrast, this manoever had no effect upon the apparent V_{max} of the system for phosphate (Fig. 4).

The effect of pH and of an inside negative diffusion potential (potassium gradient/valinomycin) upon phosphate uptake

The rate of sodium-dependent phosphate uptake in renal brush-border membranes is increased as the pH of the incubation media is raised [27]. In contrast, however, intestinal brush-border membranes show an opposite pH dependence of phosphate transport, higher at low pH values [28]. The pH profile of phosphate uptake into LLC-PK_1 apical membrane vesicles (Fig. 5) shows that increasing pH leads to a decrease in phosphate uptake rates. Assuming an absence of a pH effect upon the transporter itself, these results are com-

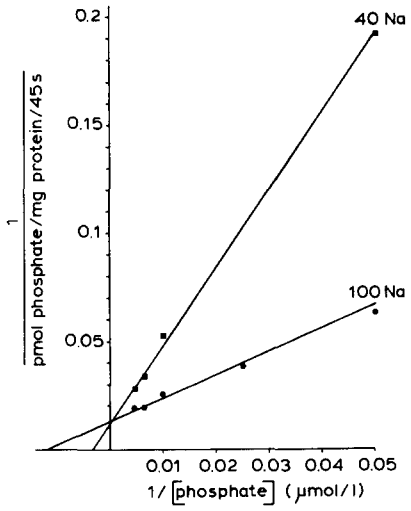

Fig. 4. Kinetic analysis of phosphate uptake at two different sodium concentrations. Uptake conditions were identical to Fig. 3a, except that in addition to the determination at 100 mmol/l Na^+ (●———●) uptake was also determined at 40 mmol/l Na^+ (choline replacement, ■———■). Lineweaver-Burk plots of this data (corrected for uptake in a Na^+-free media) gave an apparent K_m of 89.2 ± 6.1 μmol/l phosphate at 100 mmol/l Na^+ and 312 ± 12 μmol/l phosphate at 40 mmol/l Na^+. The apparent V_{max} was 79.7 ± 11 pmol/mg per 45 s. Each point represents the mean of three determinations.

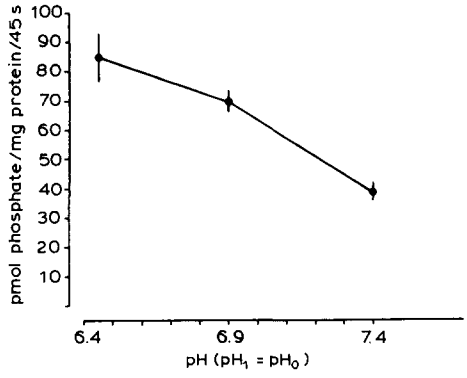

Fig. 5. The pH dependence of phosphate uptake. The vesicles contained 300 mmol/l mannitol and either 20 mmol/l Hepes-Tris at pH 6.9 or pH 7.4 or 20 mmol/l Mes-Tris at pH 6.4. The incubation media contained in addition 0.1 mmol/l KH_2PO_4 (^{32}P-labelled) and 100 mmol/l NaCl. The data are corrected for uptake observed in the presence of a 100 mmol/l KCl gradient. Phosphate uptake was measured over a 45 s uptake period and each point represents the mean ± S.D. of three determinations.

patible with the preferential transport of the monobasic form of phosphate. However, if this were the case then considering the apparent 2 : 1 coupling between Na^+ and phosphate, the transporter should be electrogenic at both pH 6.4 and pH 7.4. To test for electrogenicity, the effect of an inside negative membrane potential upon phosphate uptake rates at pH 6.4 and pH 7.4 was tested. Under conditions of pH equilibrium ($pH_o = pH_i$); vesicles were loaded with 100 mmol/l K^+ and phosphate uptake measured, under conditions of an outwardly directed K^+ gradient, in the presence and absence of valinomycin (1 μg/ml). No effect of potential upon phosphate uptake could be found, i.e. sodium-dependent phosphate uptake was electroneutral at both pH values tested.

These results can be interpreted in the following mode: The transport system accepts only dibasic phosphate and electroneutrality is the expression of a 2 : 1 coupling between sodium and dibasic phosphate. The observed pH dependence of the transport of phosphate would then be a result of a direct effect of pH upon the transporter – as is the case in intestinal and renal brush-border membranes [27,28]. Further studies will be necessary to elucidate in depth the pH dependence of phosphate transport of LLC-PK_1 membranes and to support our interpretation. It would be important to analyse the kinetics of the transport system at different pH values with respect to saturation with phosphate and sodium [27,28].

Discussion

The data presented in this report firstly describe a method for the purification of well defined apical plasma membrane vesicles from a cultured renal epithelial cell line LLC-PK_1 [3], and secondly characterizes the transport properties of these vesicles with regard to phosphate.

The membrane isolation procedure was essentially the same as has already been described for LLC-PK_1 cells [5,29] except that we chose to exploit the advantages inherent in the use of microcarrier culture techniques [12] to grow large amounts of cells. The results presented in Table I show that the final membrane pellet (P3) is enriched in the apical membrane marker enzymes: alkaline phosphatase, leucine amino peptidase and

trehalase. It is interesting to note that the specific activities of several of the enzymes (Table IB)) are at least 10-fold greater than were found by both Lever [29] and Handler and co-workers [5]. Since we use our cells after just 6 days in culture, this may represent a time, or density, dependent expression of enzymes similar to the time-dependent expression of sodium-dependent hexose transport activity [30] in this cell line.

In a previous report [8] we described the properties of a sodium-dependent phosphate transport system in intact LLC-PK_1 cells. We now present evidence to suggest that similar to other phosphate transporting epithelia [11] sodium-dependent phosphate uptake into LLC-PK_1 cells is a function of the apical membrane. A comparison of the properties of phosphate uptake into LLC-PK_1 apical membrane vesicles shows many similarities with phosphate transport into intact LLC-PK_1 cells [8] and with renal and intestinal tissue. Thus, phosphate uptake into LLC-PK_1 apical membrane vesicles is markedly dependent upon extracellular sodium ions (Fig. 1). Similar to renal and intestinal brush-border membranes [21,26] this process is very specific for Na^+. Li^+, K^+, Cs^+ and choline are ineffective replacements for Na^+. The apparent K_m for Na^+ of phosphate uptake into LLC-PK_1 apical membrane vesicles of 31.6 ± 12 mmol/l is close to that reported for renal brush-border vesicles (60 mmol/l) [24]. Similarly the apparent K_m of the system for phosphate at pH 7.4 and 100 mmol/l Na^+ is 99.2 ± 19 μmol/l. In renal brush-border membrane a K_m of between 80 and 85 μmol/l phosphate has been reported [23,24].

Arsenate has been widely used as an inhibitor of phosphate uptake by epithelia. In rat renal brush-border membrane vesicles, arsenate has been shown to act as a competitive inhibitor of phosphate uptake and to have an apparent K_i of 1.1 mmol/l [24]. The apparent K_i for arsenate inhibition in LLC-PK_1 apical vesicles was calculated to be 1.9 mmol/l. Arsenate was found not to affect the Na^+-independent component of phosphate uptake.

It has been proposed that the transport of phosphate into renal and intestinal brush-border membrane vesicles is the result of the cotransport of one phosphate with two sodium ions [21,24]. The transporter seems to accept both monobasic and dibasic phosphate. In renal brush-border membranes, at alkaline pH the system is electroneutral while at pH 6.3 the system is electrogenic [23,27]. In intestinal vesicles, electrogenicity is expressed at low pH [28]. In LLC-PK_1 apical vesicles, kinetic analysis of the dependence of phosphate uptake upon Na^+ suggests that the coupling between Na^+ and phosphate in LLC-PK_1 apical membrane vesicles is also 2 : 1, since a Lineweaver-Burk plot of $1/v$ against $1/[Na^+]$ gave a straight line and a Hill plot of the same data gave a Hill coefficient of 1.6 ± 0.2. The observation of electroneutrality of sodium-dependent phosphate transport in LLC-PK_1 cells at different pH values would suggest a preferential transport of dibasic phosphate. Electroneutrality would then be an expression of cotransport of two sodium ions with dibasic phosphate. The pH dependence would in this case not be related to the ionic form of the substrate transported (monobasic/dibasic) but would rather be an expression of the pH dependent changes of other parameters of the phosphate translocating mechanism.

Although we have not specifically addressed the question, it is interesting to see that LLC-PK_1 cells express a sodium-dependent phosphate transporter both while they are subconfluent (60–70% confluent on plates [8]) and when confluent as monolayers grown upon microcarrier beads. The data presented in this paper localises a Na^+-dependent transporter to the apical membrane at a time when these vesicles do not transport D-glucose (unpublished observations). These observations suggest that like polarised amino acid transport into these cells [31] expression of a Na^+-dependent phosphate transporter do not require the cells to be "terminally differentiated" [5,30].

Acknowledgments

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