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Effect of pH on the kinetics of Na^+-dependent phosphate transport in rat renal brush-border membranes

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The kinetics of Na^+-dependent phosphate uptake in rat renal brush-border membrane vesicles were studied under zero-trans conditions at 37°C and the effect of pH on the kinetic parameters was determined. When the pH was lowered it turned out to be increasingly difficult to estimate initial rates of phosphate uptake due to an increase in aspecific binding of phosphate to the brush border membrane. When EDTA or β-glycerophosphate was added to the uptake medium this aspecific binding was markedly reduced. At pH 6.8, initial rates of phosphate uptake were measured between 0.01 and 3.0 mM phosphate in the presence of 100 mM Na^+. Kinetic analysis resulted in a non-linear Eadie-Hofstee plot, compatible with two modes of transport: one major low-affinity system ($K_m \approx 1.3$ mM), high-capacity system ($V_{max} \approx 1.1$ nmol/s per mg protein) and one minor high-affinity ($K_m \approx 0.03$ mM), low-capacity system ($V_{max} \approx 0.04$ nmol/s per mg protein). Na^+-dependent phosphate uptake studied far from initial rate conditions i.e. at 15 s, frequently observed in the literature, led to a dramatic decrease in the V_{max} of the low-affinity system. When both the extra- and intravesicular pH were increased from 6.2 to 8.5, the K_m value of the low-affinity system increased, but when divalent phosphate is considered to be the sole substrate for the low-affinity system then the K_m value is no longer pH dependent. In contrast, the K_m value of the high-affinity system was not influenced by pH but the V_{max} decreased dramatically when the pH is lowered from 8.5 to 6.2. These results suggest that the low-affinity, high-capacity system transports divalent phosphate only while the high-affinity, low-capacity system may transport univalent as well as divalent phosphate. Raising medium sodium concentration from 100 to 250 mM increased Na^+-dependent phosphate uptake significantly but the pH dependence of the phosphate transport was not influenced. This observation makes it rather unlikely that pH changes only affect the Na^+ site of the Na^+-dependent phosphate transport system.

Introduction

Brush-border membranes of rat proximal tubules contain a Na^+-dependent phosphate transport system which has been studied extensively [1–6]. The experimental conditions chosen to characterize this system have not always been appropriate and this can partly explain the large differences in kinetic parameters reported by various groups. All studies, except one, were carried out at 25°C, at which temperature a single mode of transport is observed [2–4]. However, at 35°C a dual mechanism was reported by Brunette et al. [5]. In view of another recent publication [6] it is

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

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likely that previous studies on Na^+-dependent phosphate transport were not done under initial rate conditions.

All studies on the effect of pH on renal Na^+-dependent phosphate transport report an increase in the rate of Na^+-dependent phosphate transport when the pH is increased from 6.0 to 8.5 [2–7]. From the pH dependence, several investigators concluded that divalent phosphate is preferentially transported [2,4,5,7]. In contrast, others concluded that the pH dependence of Na^+-dependent phosphate transport reflects an inherent property of the system itself, i.e., a decrease in pH decreases the affinity of the Na^+ site for Na^+ [3,6].

Direct comparison of the results of several studies is not quite possible due to the large variation in experimental conditions, as for example the range of phosphate concentrations studied, the magnitude of the Na^+-driving force, temperature, initial rates and transmembrane pH gradients. Therefore, we reinvestigated the kinetic parameters and the effect of pH on Na^+-dependent phosphate transport in renal brush-border vesicles at 37°C under proper initial rate and zero-trans conditions.

Materials and Methods

Male Wistar-Kyoto rats (200–250 g) maintained on a normal diet (0.84% w/w P, RMH-TM, Hope Farms, Woerden, The Netherlands) were used throughout this study. Renal brush-border membranes were isolated from rat renal cortex with two subsequent Mg^{2+}-precipitation steps according to Biber et al. [8], using a Dounce apparatus with a tight-fitting pestle for homogenization. In the final isolation step, brush-border membrane vesicles were washed and resuspended in a standard medium containing 100 mM KCl, 100 mM mannitol, 1 mM NaN_3, 20 mM Tris-Hepes (pH ≥ 6.8) or 20 mM Mes-Tris (pH < 6.8). The pH of this final medium was always equal to the pH of the uptake medium, avoiding transmembrane pH gradients. Purity of brush-border membrane preparations was similar as previously reported [9].

Transport of ^{32}[P]phosphate into brush-border membrane vesicles was measured by means of a rapid filtration technique using 0.45 μm filters (Schleicher and Schüll, Dassel, F.R.G.). 8 min before the experiment, the vesicles and the uptake medium were brought to 37°C in a water bath. Incubation was started by adding 5 μl of membrane suspension (30 μg protein) to 45 μl of uptake medium containing 0.01–3 mM K_2HPO_4 (final concentration). The uptake reaction was quenched by addition of 1.1 ml of an ice-cold stop solution. The radioactivity on the filters was counted by means of conventional liquid scintillation techniques. All uptakes were carried out in triplicate at 37°C. Uptake experiments were carried out in a standard uptake medium containing 100 mM NaCl, 100 mM mannitol, 1 mM NaN_3, 20 mM Tris-Hepes (pH ≥ 6.8) or 20 mM Mes-Tris (pH < 6.8). The stop solution contained 100 mM KCl, 100 mM mannitol, 1 mM NaN_3, and 10 mM Na_2HAsO_4 buffered as the uptake medium. The Na^+-dependent phosphate uptake as a function of substrate concentration was analyzed using Eadie-Hofstee plots and evaluated according to a two-component model [10].

$$v = \frac{V_1 \cdot s}{K_1 + s} + \frac{V_2 \cdot s}{K_2 + s}$$

$$v = \frac{(V_1 \cdot K_2 + V_2 \cdot K_2)s + (V_1 + V_2)s^2}{K_1 \cdot K_2 + (K_1 + K_2)s + s^2}$$

This summation of two Michaelis-Menten equations was used to analyze the data with a non-linear least-squares regression program yielding the kinetic parameters (program designed by Dr. P. Barts).

Alkaline phosphatase activity of brush border membrane vesicles was measured at 37°C with 3 mM β-glycerophosphate as substrate added to the uptake medium (pH 8.5). The reaction was stopped by addition of 0.5 M NaOH. The amount of released phosphate was measured with a modified Fiske-SubbaRow method [11]. Protein was determined with a Coomassie blue kit (Bio-Rad, Munich, F.R.G.). The results are expressed as means ± S.E. and were evaluated statistically by unpaired Student's *t*-test.

Results

Initial rate of phosphate uptake

In order to work under initial rate conditions,

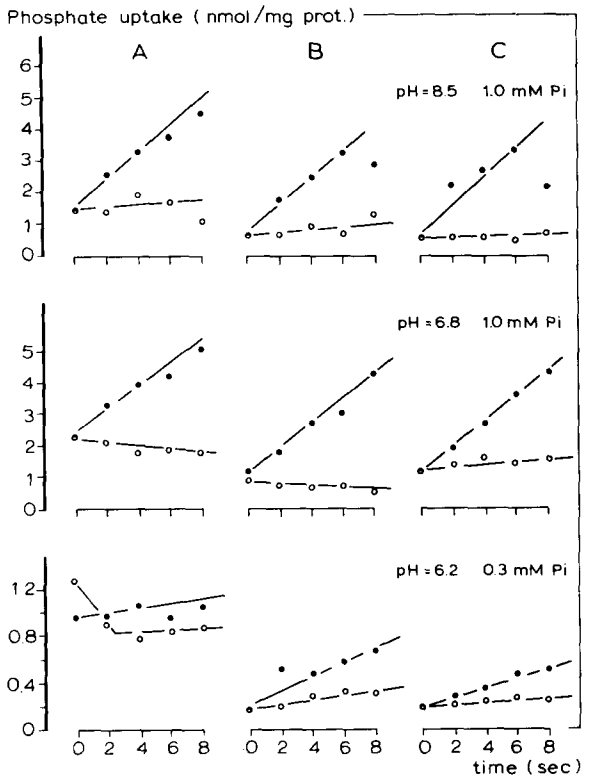

Fig. 1. Time-course of phosphate uptake at 37°C by renal brush-border membrane vesicles in the standard uptake medium in the presence of 100 mM NaCl (●) or 100 mM KCl (○). pH and phosphate (P_i) concentration as indicated. (A) Control. (B) Vesicles were loaded in the standard medium in the presence of 0.5 mM EDTA and 3 mM β-glycerophosphate. (C) Vesicles loaded in the presence of 0.5 mM EDTA. Values of a typical experiment in triplicate are given. The results are essentially similar for all phosphate concentrations tested.

the rate of phosphate uptake was studied at three pH values at several phosphate concentrations. A typical set of results is shown in Fig. 1. In fresh preparations it turned out to be increasingly difficult to determine phosphate uptake rates when the pH was lowered (Fig. 1A). At pH 6.2 more phosphate was 'bound' when K^+ was present than with Na^+ present. This phosphate binding phenomenon could not be reduced by filtering or boiling the uptake medium. However, with 3 mM β-glycerophosphate and 0.5 mM EDTA (Fig. 1B) or 0.5 mM EDTA alone (Fig. 1C) in the uptake medium most of the binding disappeared and, more important, the rate of phosphate uptake appeared linear up to 4 or 8 s, depending on the pH and phosphate concentration tested.

Fig. 2 shows that alkaline phosphatase, present in brush-border membranes, hydrolyses β-glycerophate thereby releasing inorganic phosphate. Alkaline phosphatase activity was inhibited only 25% with 0.5 mM EDTA. However, when brush borders were preincubated with 0.5 mM EDTA for 1 h on ice, the alkaline phosphatase activity was almost completely inhibited. Since very low phosphate concentrations were used, it is important to avoid release of inorganic phosphate. In addition it has been shown by Letelier et al. [12] that P_i released from β-glycerophosphate is 10-times faster transported into brush-border vesicles than medium P_i. Therefore the effect of β-glycerophosphate was tested on the initial rate of Na^+-dependent phosphate uptake at three different pH values. Addition of 3 mM β-glycerophosphate has no significant effect on the rate of P_i uptake at pH 8.5 when 0.5 mM EDTA is also present. However at pH 6.8 and 6.2 there is a small but significant increase in initial rate of P_i uptake.

Therefore, all P_i uptake were done in the presence of 0.5 mM EDTA and in the absence of 3 mM β-glycerophosphate. Previously it has been shown that EDTA does not influence Na^+-dependent phosphate transport, while it inhibits for 90% alkaline phosphatase activity [13,14].

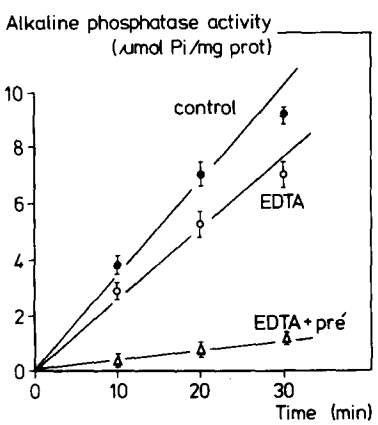

Fig. 2. Alkaline phosphatase activity as a function of incubation time in renal brush-border membrane vesicles at 37°C with 3 mM β-glycerophosphate as substrate. Experiments were carried out without (●) and with (○, △) 0.5 mM EDTA present in the standard uptake medium. (△) Brush-border membrane vesicles were preincubated for 1 h on ice with 0.5 mM EDTA. Results are means ± S.E. of experiments in triplicate on four different membrane preparations.

TABLE I

EFFECT OF β-GLYCEROPHOSPHATE ON PHOSPHATE UPTAKE IN RENAL BRUSH-BORDER MEMBRANE VESICLES AT 37°C

The phosphate uptake was measured at 4 s in the presence of 100 mM Na^+ or 100 mM K^+ with or without 3 mM β-glycerophosphate. The standard incubation medium was used with 0.5 mM EDTA. Brush-border membrane vesicles were loaded with standard buffer in the presence of 0.5 mM EDTA. Results are means of uptake rates obtained in triplicate in five different membrane preparations. ($n = 5$; $\bar{x} \pm$ S.E.). v, Na^+-dependent phosphate uptake; P, significance between − and + β-glycerophosphate.

pH	P_i (mM)	β-Glycero-phosphate	Phosphate uptake (nmol/s per mg protein)		
			Na^+	K^+	v
6.2	0.1	−	0.037 ± 0.001	0.014 ± 0.001	0.023 ± 0.003
		+	0.049 ± 0.005	0.013 ± 0.001	0.033 ± 0.004
			$P < 0.03$ *	$P > 0.45$	$P < 0.06$
6.2	3.0	−	0.63 ± 0.01	0.29 ± 0.02	0.35 ± 0.04
		+	0.76 ± 0.05	0.27 ± 0.04	0.45 ± 0.04
			$P < 0.03$ *	$P > 0.75$	$P < 0.05$ *
6.8	0.1	−	0.11 ± 0.01	0.016 ± 0.003	0.10 ± 0.01
		+	0.13 ± 0.01	0.015 ± 0.002	0.12 ± 0.01
			$P < 0.008$ *	$P > 0.75$	$P < 0.02$ *
6.8	3.0	−	1.03 ± 0.10	0.52 ± 0.06	0.50 ± 0.06
		+	1.10 ± 0.06	0.43 ± 0.04	0.67 ± 0.06
			$P > 0.55$	$P > 0.23$	$P > 0.07$
8.5	0.1	−	0.17 ± 0.02	0.020 ± 0.002	0.15 ± 0.02
		+	0.17 ± 0.02	0.020 ± 0.003	0.15 ± 0.01
			$P > 0.92$	$P > 0.99$	$P > 0.99$
8.5	3.0	−	0.78 ± 0.09	0.38 ± 0.04	0.40 ± 0.09
		+	0.93 ± 0.13	0.41 ± 0.06	0.53 ± 0.09
			$P > 0.30$	$P > 0.60$	$P > 0.29$

* $P < 0.05$.

Kinetics of Na^+-dependent phosphate uptake

Initial rates of phosphate uptake (4 s) were studied with phosphate concentrations varying between 0.01 and 3 mM. An Eadie-Hofstee plot of Na^+-dependent phosphate uptake at pH 6.8 is given in Fig. 3 (left panel). A deviation from linearity or single Michaelis-Menten kinetics is observed, indicative of a dual mechanism or a two-site transport mechanism for phosphate in the brush-border membrane. Brunette et al. [5] also reported a second component in Lineweaver-Burk plots of Na^+-dependent phosphate uptake when phosphate uptake was studied at 35°C. We fitted our data to a two-site mechanism by non-linear least-squares regression and calculated the maximal transport capacity, V_{max}, and the apparent affinity constant, K_m, for the high and low affinity system. The K_m values are 27 and 1350 μM while the V_{max} values are 0.04 and 1.10 nmol/s per mg protein for the high- and low-affinity system, respectively (Table II).

When Na^+-dependent phosphate uptake is studied under non-initial rate conditions at 15 s the V_{max} values decrease to 0.03 and 0.20 nmol/s per mg protein, respectively (Fig. 3, right panel). In this situation, K_m values are 8 and 666 μM. Hence, non-initial rate conditions affect in particular the V_{max} of the low-affinity system and not the K_m values of both systems.

pH-dependence of Na^+-dependent phosphate uptake

The initial rate of phosphate uptake in the presence of Na^+ and 0.1 mM phosphate increased sharply by raising the pH from 6.2 to 6.8 (Fig.

TABLE II

KINETIC PARAMETERS OF Na^+-DEPENDENT PHOSPHATE UPTAKE IN RENAL BRUSH-BORDER MEMBRANE VESICLES AT 37°C

Kinetical parameters were derived from the Eadie-Hofstee plots in Figs. 3A, 5, and 6A. The subscripts 1 and 2 refer to the high- and low-affinity system. K'_m and K''_m are calculated using either the monovalent or divalent phosphate concentrations which are present at the three pH values ($pK = 6.8$ at 37°C). When individual experiments are fitted and the resulting K_m and V_{max} values are averaged, the S.E. values for K_m are within 20% of the mean and the S.E. values for V_{max} are within 5% of the mean.

pH	μM						nmol/s per mg protein		Corr. coeff. of computer fit
	K_m^1	K_m^2	$K_m'^1$	$K_m'^2$	$K_m''^1$	$K_m''^2$	V_{max}^1	V_{max}^2	
8.5	27	453	1	9	26	444	0.16	0.48	0.999
6.8	27	1350	14	675	14	675	0.04	1.10	0.999
6.2	2	1976	1.6	1581	0.4	395	0.001	0.59	0.998

4A). A further raise in the pH to 8.5 stimulated phosphate uptake only slightly. The same pattern of pH-dependence was observed at 25°C (data not shown). At 3 mM phosphate, the initial rate of phosphate uptake in the presence of a Na^+ gradient was also enhanced by a pH rise from 6.2 to 7.1

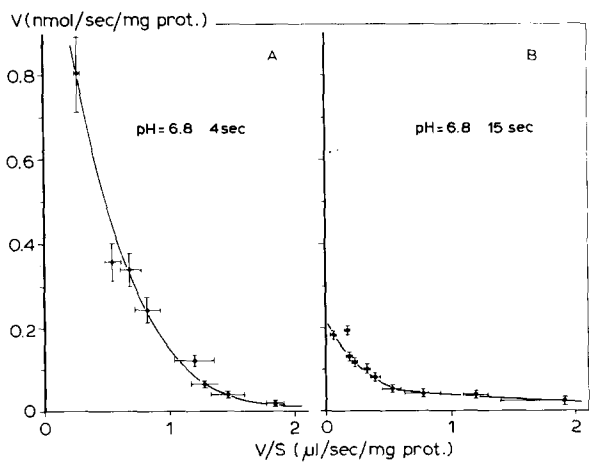

Fig. 3. Eadie-Hofstee plots of Na^+-dependent phosphate uptake in renal brush-border membrane vesicles at 37°C. The left panel shows data obtained under initial rate conditions (4 s) while the right panel gives values obtained with 15-s uptake points. Phosphate concentrations varied between 0.01 and 3.0 mM. Brush-border membrane vesicles were loaded in standard medium (pH 6.8) in the presence of 0.5 mM EDTA. The uptake medium (pH 6.8) also contained 0.5 mM EDTA. The data have been corrected for Na^+-independent phosphate uptake in the presence of 100 mM KCl instead of NaCl. The lines represent a non-linear regression of the data, used to calculate the kinetical constants (correlation coefficient of the fit is 0.999). Results are means of uptake rates of experiments in triplicate on four different membrane preparations.

(Fig. 4B). In contrast to Fig. 4A a further increase in the pH reduced phosphate uptake. Therefore, the Na^+-dependent phosphate cotransporter displays a pH optimum between pH 7.1 and pH 7.4, when measured under V_{max} conditions. Na^+-independent phosphate uptake was not influenced by pH at both phosphate concentrations studied. The

TABLE III

THE EFFECT OF 250 mM Na^+ ON THE pH-DEPENDENCE OF THE Na^+-DEPENDENT PHOSPHATE UPTAKE IN RENAL BRUSH-BORDER MEMBRANE VESICLES AT 37°C

The phosphate uptake (nmol/s per mg protein) was measured at 4 s in the presence of 100 or 250 mM NaCl and mannitol to keep the osmolarity constant at 500 mosM. The phosphate uptake was corrected for the Na^+-independent phosphate uptake determined in the presence of 100 or 250 mM KCl instead of NaCl in the uptake medium. Brush-border membrane vesicles were loaded with a medium containing 500 mM mannitol, 1 mM NaN_3, 0.5 mM EDTA, 20 mM Mes-Tris (pH 6.4) or 20 mM Tris-Hepes (pH 7.4). Results are means ± S.E. of uptake rates obtained in triplicate in five different membrane preparations ($n = 5$; $\bar{x} \pm$ S.E.). P, significance of difference between 100 and 250 mM Na^+.

P_i (mM)	Na^+ (mM)	pH	
		6.4	7.4
0.1	100	0.034 ± 0.009	0.120 ± 0.013
	250	0.154 ± 0.034	0.308 ± 0.046
		$P < 0.005$ *	$P < 0.002$ *
3.0	100	0.278 ± 0.036	0.329 ± 0.025
	250	0.426 ± 0.036	0.473 ± 0.033
		$P < 0.01$ *	$P < 0.005$ *

* $P < 0.01$.

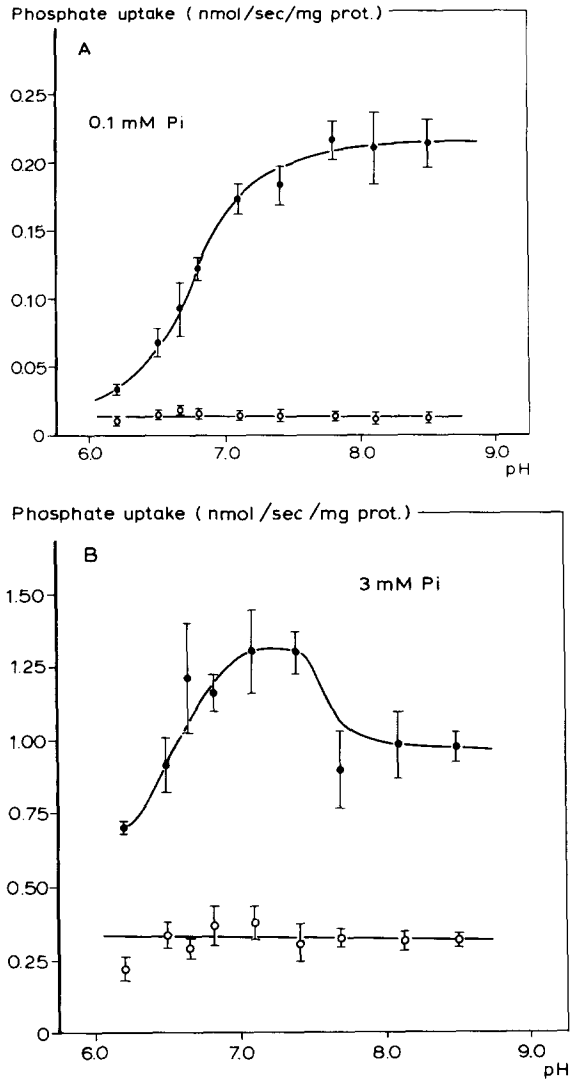

Fig. 4. pH dependence of initial rate of phosphate uptake by renal brush border membrane vesicles at 37°C in the presence of 100 mM NaCl (●) or 100 mM KCl (○). Phosphate (P_i) concentrations used were 0.1 mM (A) and 3 mM (B). Brush-border membrane vesicles were loaded in standard medium in the presence of 0.5 mM EDTA. The uptake medium contained 0.5 mM EDTA. Results are means of uptake rates of experiments in triplicate (4 s of incubation) on five different membrane preparations.

great S.E. values shown in Fig. 4B resulted from large differences between various brush-border preparations rather than from scatter in phosphate uptake values within one single experiment. With every brush-border preparation the whole pH range was studied and the typical pH dependence

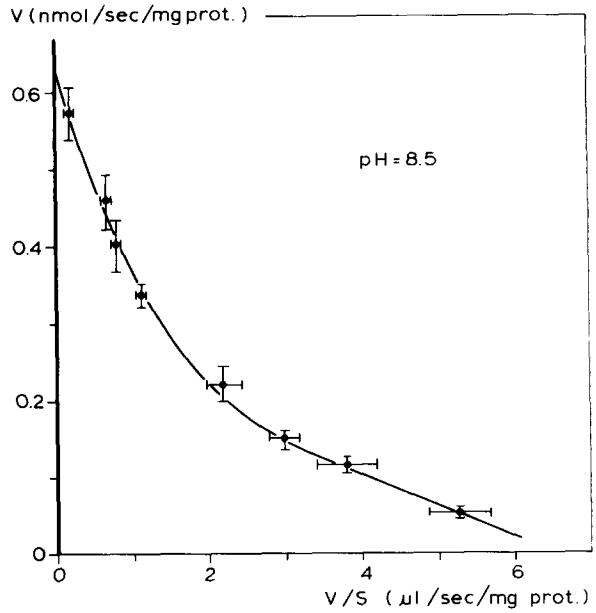

Fig. 5. Eadie-Hofstee plot of Na^+-dependent phosphate uptake in renal brush-border membrane vesicles at 37°C and pH 8.5 (4 s uptake points). Results are means of uptake rates of experiments in triplicate on four different membrane preparations. Uptake conditions as described in the legend of Fig. 3 (correlation coefficient of the fit is 0.999).

was seen in every single preparation.

Na^+-dependent phosphate uptake as a function of phosphate concentration was also investigated

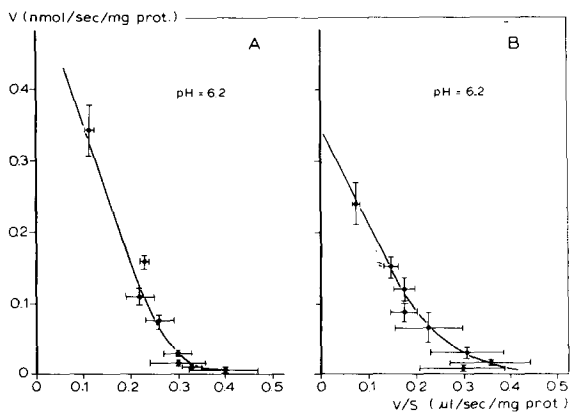

Fig. 6. Eadie-Hofstee plot of Na^+-dependent phosphate uptake in renal brush-border membrane vesicles at 37°C and pH 6.2 (8-s uptake points). Uptake in the presence of 0.5 mM EDTA (panel A) and 0.5 mM EDTA + 3 mM β-glycerophosphate (panel B). Results are means of uptake rates of experiments in triplicate on three different preparations (correlation coefficient of the fit is 0.998).

at pH 8.5 and 6.2 (Figs. 5 and 6A). At both extreme pH values, a dual Na^+-dependent phosphate transport system was apparent and when the data were fitted to a two-site system a high- and a low-affinity system emerged. Since at low pH β-glycerophosphate stimulated Na^+-dependent P_i transport, the kinetics were also studied in the presence of β-glycerophosphate (Figs. 6A and 6B). Comparison of Figs. 6A and 6B suggests that β-glycerophosphate specifically stimulates the high-affinity system, since in Fig. 6B the high-affinity system is more pronounced present than in Fig. 6A. The calculated kinetic parameters from Eadie-Hofstee plots at pH 6.2, 6.8 and 8.5 are listed in Table II. The K_m of the low affinity system increased dramatically with decreasing pH. Assuming monovalent phosphate as substrate for the carrier the rise in K'_m became even more pronounced. When divalent phosphate is assumed to be the sole substrate the K''_m value appears independent of the pH. This result suggests that the low-affinity system preferentially transports divalent phosphate. It appears that the K_m value of the high-affinity system is not influenced significantly by pH except at pH 6.2. Apparently, there is no preferred phosphate ion for the high-affinity system. The V_{max} of the high-affinity system decreased strongly with decreasing pH values and the high-affinity system is almost undetectable at acid pH. The V_{max} of the low-affinity system is highest at pH 6.8 and lower at pH 8.5 and 6.2, which is in agreement with the data presented in Fig. 4B. Since the pH-induced changes in V_{max} cannot be explained by a preferential uptake of one ionic species, these changes in V_{max} must reflect an influence of pH on the transport system itself.

Amstutz et al. [6] argued that lowering the pH decreases the affinity for Na^+ of the Na^+-dependent phosphate transport system. They showed that increasing the Na^+ concentration reduced the pH effect on Na^+-dependent phosphate uptake. We studied phosphate uptake at 100 and 250 mM Na^+ at pH 6.4 and 7.4 both at low and high phosphate concentrations. The results are shown in Table III. Increasing Na^+ concentration from 100 to 250 mM increased Na^+-dependent phosphate uptake significantly at the two phosphate concentrations tested. Most important, the pH effect on Na^+-dependent phosphate uptake is not substantially altered by the increase in Na^+ concentration.

Discussion

The kinetics of Na^+-dependent phosphate transport in rat renal brush-border vesicles were studied at 37°C under initial rate conditions over a wide range of phosphate concentrations. Our study revealed two phosphate transport systems, a major low affinity and a minor high affinity system. The effects of pH on both systems can be explained best when it is assumed that the high capacity system transports divalent phosphate preferentially while the low capacity system transports monovalent as well as divalent phosphate.

It is firmly established that phosphate transport in the kidney is coupled to sodium and is strongly influenced by pH [15,16]. Uncertainty exists about the kinetic parameters of Na^+-dependent phosphate transport as can be seen in Table IV, where reported kinetic parameters are listed. The large variation in K_m and V_{max} values probably arises from different experimental conditions used by the various authors.

The first condition of importance is the ambient temperature. At 37°C a dual mechanism is always observed but at 25°C the high affinity system is less conspicuous. At 25°C a slight deviation from linear Michaelis-Menten kinetics can be observed [9]. The occurrence of two systems can partly explain the variation in K_m values (Table IV). Depending on the range of phosphate concentrations used, K_m values between 30 and 1300 μM can be expected.

The second condition of importance is initial rate. Most values in Table IV were obtained from either 15-s or 30-s uptake points, which is far from the initial rate [6]. We have shown that non-initial rate conditions especially effects the V_{max} of the low-affinity system (Fig. 3), making the high affinity relatively more important. This effect is expressed in Table IV where the relatively long uptake points yield the lowest K_m values.

The third condition is the pH value at which the kinetics are studied. At physiological pH values inorganic phosphate is present in two forms: $H_2PO_4^-$ and HPO_4^{2-}. The question which form is

TABLE IV

KINETIC PARAMETERS OF Na^+-DEPENDENT PHOSPHATE UPTAKE IN RENAL BRUSH-BORDER MEMBRANE VESICLES

All studies used a buffered uptake medium containing 100 mM NaCl, except Amstutz et al. [6] who used at pH 6.4 130 mM and at pH 7.4 120 mM NaCl. Time, uptake time; °C, uptake temperatures; P_i range, studied concentration range of phosphate; RT, room temperature; ?, not reported; *, Table II (own observations).

Time (s)	°C	Species	pH_0	K_m (μM)	V_{max} (nmol/min per mg protein)	P_i range (μM)	Ref.
10	20	rabbit	7.3	136	5.5	25–1000	7
20	20	rabbit	7.5	85	5.4	25–1000	4
30	20	rabbit	7.5	53	2.7	50–1000	17
20	?	dog	7.5	51	4.8	10– 500	18
20	?	dog	7.5	51	4.8	10–2000	19
30	?	dog	8.5	68	15.0	50–1000	20
10	25	rat	6.3	627	11.2	100–1500	3
< 7	RT	rat	6.4	954	6.7	50–1000	6
10	25	rat	6.9	1464	49.7	100–1500	3
15	RT	rat	7.4	190	13.0	10–1000	21
10	25	rat	7.4	1392	85.0	100–1500	3
15	25	rat	7.4	80	65.0	500–4000	2
< 7	RT	rat	7.4	203	8.0	50–1000	6
15	25	rat	7.4	57	9.6	30–1000	9
15	22	rat	7.5	130	8.5	10–5000	23
10	25	rat	7.8	1364	77.9	100–1500	3
15	22	rat	8.5	36	12.5	20–1000	24
10	25	rat	8.5	89	23.2	40– 300	5
30	20	rat	8.5	72	5.0	50–1000	17
30	?	rat	8.5	95	8.3	50–1000	25
30	20	rat	8.5	62	12.4	50–1000	26
8	35	rat	6.2	2–1976	0.06–36	10–3000	*
10	35	rat	6.5	341	25.8	40– 300	5
4	35	rat	6.8	27–1350	2.4–66	10–3000	*
10	35	rat	7.5	167	33.0	40– 300	5
10	35	rat	8.5	94	19.8	40– 300	5
10	35	rat	8.5	3–1610	21–30	40–3000	26
4	35	rat	8.5	27– 453	9.6–30	10–3000	*

preferentially transported has been asked by various investigators. However, these studies are never unambiguous, since variations in the ratio monovalent/divalent phosphate can only be realized by varying the pH. This may directly affect the kinetic parameters of Na^+-dependent phosphate transport. Most studies agree on preferential transport of divalent phosphate [2,4,5,7]. In order to evaluate the effect of pH on Na^+-dependent phosphate transport, the two transport systems have to be considered separately because pH changes affect both systems in a different way. Considering firstly the major low affinity system, we conclude that divalent phosphate is the prefered substrate. The apparent K''_m for divalent phosphate appears not to be influenced by pH (Table II). The same conclusion was reached by Rabito et al. [28] from studies on the pH dependence of phosphate uptake at 37°C by a kidney cell line. Our conclusion is further strengthened by the results shown in Fig. 4A. Na^+-dependent phosphate uptake increased sharply between pH 6.2 and 7.0 when uptake rates are studied at phosphate concentrations well below the K_m value. Apparently the

low concentration of divalent phosphate below pH 6.8 becomes a limiting factor. Also in micropuncture studies on chronic parathyroidectomized rats, it was shown that phosphate reabsorption decreased below pH 6.8 and no further increase was found between pH 6.7 and 7.8 [29].

An alternative explanation for the effect of pH on the K_m for phosphate is favored by Burckhardt et al. [3] and Amstutz et al. [6]. The reasoning is that lowering the pH decreases the Na^+ affinity of the phosphate transporter. In their study an increase in external Na^+ concentration counteracted partly the inhibition of Na^+-dependent phosphate transport at lower pH values .Nevertheless, even at saturating Na^+ concentrations (300 mM) a decrease in pH increased the K_m value for phosphate. In our study, the effect of pH on Na^+-dependent phosphate transport was more or less independent of the ambient Na^+ concentration. At 250 mM Na^+ the phosphate transport rates were higher but the relative effect of pH was similar as with 100 mM Na^+ (Table III). Therefore, at phosphate concentrations around the K_m value or lower, the pH dependence merely reflects the preference for divalent phosphate. It can be anticipated that at higher phosphate concentrations, when at every pH value maximal transport rates are measured, an effect of pH on the carrier will be observed. This experiment is shown in Fig. 4B. The V_{max} of the low-affinity system was maximal between pH 7.1 and 7.4. Phosphate reabsorption in isolated perfused proximal tubules of the rabbit was also maximal at neutral pH and high phosphate concentration [30]. The mechanism behind the pH effect on the V_{max} is not known but it probably reflects a pH optimum of the transport protein itself as is frequently observed for enzymes. The effect of pH on the kinetics of the high affinity system is quite different. Changes in pH primarily effect the V_{max} but not the apparent K_m.

The two Na^+-dependent phosphate transport systems observed in our study may result from tubular heterogeneities in the ability to reabsorb phosphate either along the length of proximal tubules or between cortical and juxtamedullary nephrons. It was demonstrated before that glucose carriers have different affinities and transport capacities dependent on the localization along the length of proximal tubules [31,32]. Depending on the parathyroid hormone and phosphate states, lower and higher capacities for phosphate reabsorption in deep compared to superficial nephrons have been reported [33–35]. In two recent studies, conflicting differences in V_{max} values for Na^+-dependent phosphate transport were reported between cortical and juxtamedullary brush-border vesicles [20,27]. The explanation may be the different experimental conditions used by the two groups. Therefore, further studies are required to delineate these possible tubular heterogeneities.

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