Effect of arginine modification on kidney brush-border-membrane transport activity

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The effect of phenylglyoxylation on brush-border-membrane functions was studied with membrane vesicles from rat kidney cortex. Na^+ -gradient-dependent uptake of phosphate, glucose and alanine was inhibited by 65, 88 and 70% by pre-incubation of vesicles with 50 mM-phenylglyoxal for 2 min. The inhibition showed a dependency for alkaline pH. Borate co-operativity in butanedione inactivation was used to prove that inhibition was caused by arginine modification. Intravesicular volumes, alkaline phosphatase, aminopeptidase M and Na^+ - H^+ exchange were not affected by phenylglyoxal treatment. Inhibition of phosphate uptake was studied in more detail and showed that the chemical modification introduced by phenylglyoxal inhibited the overshoot of phosphate uptake caused by the Na^+ gradient, and decreased the apparent maximal velocity of the phosphate-transport system in its interaction with Na^+ . Phosphate uptake measured in the absence of Na^+ was not affected by phenylglyoxal. Shunting of the transmembrane electrical potential with K^+ and valinomycin had no effect on phenylglyoxal inhibition, proving that the alteration of transmembrane electrical potential could not be responsible for this effect. Phenylglyoxal had no ionophoric effect on the Na^+ gradients studied (1–100 mM). Na^+ efflux was also unaffected by phenylglyoxal treatment. Na^+ , harmaline and amiloride were ineffective in protecting against phenylglyoxal inhibition, suggesting that the site modified was not an Na^+ -binding site. These results indicate the involvement of highly reactive arginine residues in phosphate, glucose and alanine uptake.

It is now well established that active solute transport in animal cells is driven by the electrochemical potential gradient of Na^+ ions (Schultz & Curran, 1970; Heinz *et al.*, 1975); numerous studies with renal and intestinal brush-border-membrane vesicles have demonstrated a coupling between Na^+ and sugar fluxes (Hopfer *et al.*, 1973; Aronson & Sacktor, 1974), amino acids (McNamara *et al.*, 1976; Hammerman & Sacktor, 1977) and phosphate (Heinz *et al.*, 1972; Hoffman *et al.*, 1976) in these organs. Mutual interactions for transport between these different solutes have been reported (Genel *et al.*, 1971; Thierry *et al.*, 1981) and seem best explained by the competition between substrates for the common driving force,

the Na^+ electrochemical gradient. The precise molecular mechanisms involved in the coupling of Na^+ and solute fluxes are still unresolved. The understanding of these mechanisms will be of crucial importance in the exact definition of the Na^+ -symport systems. Very few studies have been directed towards a search for functional groups on residues indispensable for carrier function. Thiol groups (Thomas, 1973) and tyrosine residues (Lin *et al.*, 1972) have been implicated in Na^+ -dependent glucose transport; in intestinal brush-border-membrane vesicles, amino groups have been identified as involved in glucose uptake (Weber & Semenza, 1983; Biber *et al.*, 1983). In the present paper, the involvement of arginine residues in the Na^+ -dependent uptake of phosphate, alanine and glucose in renal brush-border-membrane vesicles is reported.

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Materials and methods

Materials

Phenylglyoxal, methylglyoxal, butanedione, L-leucine, *p*-nitroaniline and *p*-nitrophenyl phosphate were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). D-[^{14}C]Glucose (specific radioactivity 15 mCi/mmol), L-[^{14}C]alanine (specific radioactivity 150 mCi/mmol), $^{22}\text{NaCl}$ (carrier-free) and [^{32}P]orthophosphate (carrier-free) were obtained from New England Nuclear (Boston, MA, U.S.A.). Other chemicals were of the highest purity commercially available.

Preparation of brush-border membranes from rat kidney cortex

Brush-border membranes were prepared as described by Booth & Kenny (1974). The purified brush-border-membrane vesicles were resuspended to a final protein concentration of 10 mg/ml in 10 mM-Tris/Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer, pH 7.5, containing 300 mM-mannitol. The enrichments in alkaline phosphatase and $\text{Na}^+ + \text{K}^+$ -dependent ATPase were 12.2 ± 0.3 - and 0.41 ± 0.1 -fold (means $\pm$ S.E.M.) respectively.

Uptake studies

The rapid-filtration technique of Hopfer *et al.* (1973) was used for uptake studies. Incubation media contained, in a 45 μl final volume: 10 mM-Tris/Hepes buffer, pH 7.5, 100 mM-mannitol, 100 mM-NaSCN or -KSCN, and 100 μM -D-[^{14}C]glucose (0.1 μCi) or 100 μM -L-[^{14}C]alanine (0.1 μCi) or 200 μM -[^{32}P]orthophosphate (4 μCi). Na^+ uptake was measured in 20 mM-Tris/Hepes buffer, pH 7.5, containing 100 mM-mannitol and indicated amounts of $^{22}\text{NaSCN}$.

Uptake studies, performed in triplicate at 25°C, were initiated by the addition of 50–70 μg of protein. After incubation, the reactions were stopped by addition of 1 ml of cold stop solution. The glucose and alanine stop solutions contained 5 mM-Tris/Hepes buffer, pH 7.5, 150 mM-NaCl and 1 mM-phlorrhizin; the phosphate stop solution contained 5 mM-Tris/Hepes buffer, pH 7.5, 100 mM-mannitol, 100 mM-choline chloride and 10 mM- Na_3AsO_4 ; the Na^+ -uptake stop solution was 150 mM-LiCl and 5 mM-Tris/Hepes buffer, pH 7.5. The suspension was applied to 0.45 μm -pore-size Millipore filters under vacuum. Filters were washed with 8 ml of ice-cold stop solution and then processed for liquid-scintillation counting. Uptake values were corrected for non-specific absorption by vesicles and filters in a separate assay where vesicles and incubation medium were directly added to the stop solution. Non-specific binding never exceeded 1.1% of normal uptake

values. Na^+ -dependent uptake was calculated as the difference between uptake values in NaSCN and KSCN. When not indicated, standard errors were less than 10% of the mean.

Pre-incubation of membrane vesicles with arginine modifiers

Membrane vesicles were treated at 25°C for various periods of time by different concentrations of phenylglyoxal, methylglyoxal or butanedione as indicated in the legends to the Figures. Transport was then measured as described above.

Enzyme activities

Alkaline phosphatase, aminopeptidase M and $\text{Na}^+ + \text{K}^+$ -dependent ATPase activities in membrane vesicles were assayed by standard procedures (Kelly & Hamilton, 1970; Berner & Kinne, 1976; Hanse *et al.*, 1978) after a 2 min incubation at 25°C in 10 mM-Tris/Hepes buffer, pH 7.5, containing 300 mM-mannitol with or without 50 mM-phenylglyoxal.

Results

Effect of phenylglyoxal on phosphate, glucose and alanine uptake

Membrane vesicles were pre-incubated at 25°C with different concentrations of phenylglyoxal for various periods of time before determination of the phosphate uptake (Fig. 1). Phosphate uptake after pre-incubation for 2 min was inhibited by 5, 37, 46, 65 and 71% at 10 mM-, 20 mM-, 30 mM-, 40 mM- and 50 mM-phenylglyoxal respectively. The effect of pre-incubation of vesicles at 50 mM-phenylglyoxal on glucose and alanine transport is shown in Figs. 2 and 3. Alanine uptake is inhibited by 70% and glucose uptake by 88% after 2 min of pre-incubation.

Effect of other arginine modifiers on phosphate uptake

Membrane vesicles were pre-incubated at 25°C with 30 mM-butanedione, -methylglyoxal or -phenylglyoxal before determination of phosphate uptake (Fig. 4). Phenylglyoxal is the strongest inhibitor, followed by methylglyoxal and butanedione, a 60 min pre-incubation giving inhibition of 82, 58 and 25% respectively. Incubation at 25°C by itself had no effect on phosphate uptake. Borate co-operativity in butanedione reactivity towards arginine residues is a well-documented fact (Riordan, 1979) and is an indication that an arginine residue has been modified. Inhibition of phosphate uptake by butanedione was seen only in the presence of borate buffer (Table 1): 49% inhibition compared with 8% in Tris/Hepes buffer. Furthermore, the inhibition seen in borate buffer is

Fig. 1. *Inhibition of phosphate uptake by brush-border-membrane vesicles by pre-incubation with phenylglyoxal* Membrane vesicles were pre-incubated in 300mM-mannitol/10mM-Tris/Hepes buffer, pH 7.5, with the following concentrations of phenylglyoxal: ●, none; ○, 10 mM; ■, 20 mM; □, 30 mM; ▲, 40 mM; △, 50 mM. Vesicles were then diluted in the phosphate-uptake medium, and uptake was measured at 20 s, as described in the Materials and methods section. Values are means $\pm$ S.D. for six experiments.

Fig. 2. *Effect of pre-incubation with phenylglyoxal on Na⁺-dependent D-glucose uptake by brush-border-membrane vesicles*

Membrane vesicles were pre-incubated in 300mM-mannitol/10mM-Tris/Hepes buffer, pH 7.5, with (○) or without (●) 50mM-phenylglyoxal, and D-glucose uptake was measured at 20 s, as described in the Materials and methods section.

partially reversible in Tris/Hepes buffer (22%) after washing of borate. Stabilization of the reaction produced by borate is another indication for arginine modification (Riordan, 1979).

Effect of pH on phenylglyoxal inactivation of phosphate transport

The inactivation of phosphate transport is influenced by the pH at which pre-incubation is carried out. The data presented in Fig. 5 show that phosphate uptake in rat brush-border-membrane vesicles is maximal at pH 8.5, in agreement with previously reported results (Cheng & Sacktor, 1981). The inhibition increases progressively from pH 6.5 to 10.5. This pH-dependency of the inhibition of phenylglyoxal is another indication that the arginine modification is responsible for this effect, as reaction of phenylglyoxal with arginine is strongly dependent on alkaline pH (Riordan, 1979).

Effect of phenylglyoxal on Na⁺-dependent phosphate uptake

Fig. 6 shows that a 2 min pre-incubation of membrane vesicles with 50mM-phenylglyoxal inhibits the overshoot usually observed when a Na⁺

gradient (outside>inside) is imposed on the vesicles. The phosphate uptakes at equilibrium state (40min) are identical in phenylglyoxal-treated and control vesicles. The effect of the magnitude of the driving force is shown in Fig. 7. Na⁺/phosphate co-transport is saturated with Na⁺ at about 100mM, the apparent *K_m* for Na⁺ being

52mM. Phenylglyoxal inhibition of phosphate uptake increases progressively as the Na⁺ concentration is raised to 30mM, but remains at a constant level of 65% inhibition as the concentration increases from 30 to 100mM (Fig. 7, inset). The relationship between phosphate uptake and Na⁺ concentration is clearly non-hyperbolic. A plot of phosphate uptake against phosphate uptake/[Na⁺]² gives straight lines (Fig. 8), suggesting

Fig. 3. Effect of pre-incubation with phenylglyoxal on Na⁺-dependent L-alanine uptake by brush-border-membrane vesicles

Membrane vesicles were pre-incubated as indicated in Fig. 2 legend with (○) or without (●) 50mM-phenylglyoxal, and alanine uptake was measured at 20s, as described in the Materials and methods section.

Fig. 4. Effect of arginine modifiers on Na⁺-dependent phosphate uptake by brush-border-membrane vesicles

The vesicles were suspended in either 100mM-mannitol/100mM-borate/KOH buffer, pH 7.5, with (■) 30mM-butanedione or 300mM-mannitol/10mM-Tris/Hepes buffer, pH 7.5, with 30mM-methylglyoxal (△) or 30mM-phenylglyoxal (○) or without either (●). The vesicles were pre-incubated for the indicated times and uptake was measured as described in the Materials and methods section. Results are expressed as the percentage of activity remaining after treatment compared with control, pre-incubated in buffer alone.

Table 1. Borate co-operativity and reversibility of butanedione inhibition of phosphate uptake by brush-border-membrane vesicles

Membrane vesicles were pre-incubated for 2min at 25°C in the indicated conditions. They were then washed by centrifugation and incubated for 90min at 25°C in the conditions described.

	Phosphate uptake (pmol/20s per µg of protein)		Inhibition (%)
	No butanedione	50mM-Butanedione	
A. Pre-incubation in Tris/Hepes, pH7.5			
+ washing and incubation for 90min at 25°C in Tris/Hepes, pH7.5	1.965 ± 0.081	1.804 ± 0.176	8 ± 7
	1.987 ± 0.192	1.875 ± 0.118	6 ± 4
B. Pre-incubation in borate/KOH, pH7.5			
+ washing and incubation for 90min at 25°C in borate/KOH, pH7.5	2.046 ± 0.160	1.043 ± 0.140	49 ± 9
+ washing and incubation for 90min at 25°C in Tris/Hepes, pH7.5	2.735 ± 0.167	1.123 ± 0.176	59 ± 4
	2.031 ± 0.155	1.554 ± 0.379	22 ± 5

that 2 Na⁺ ions interact with the transport system (Cheng & Sacktor, 1981). The main effect of phenylglyoxal on phosphate uptake appears to be on maximal velocity, the modification causing a decrease in this value from 1.35 pmol/5s per μ g of protein to 0.48 pmol/5s per μ g of protein. The affinity of the transport system for Na⁺ after

phenylglyoxal modification remains much the same (52 and 48 mM).

Effect of phenylglyoxal on other membrane functions

To clarify further the action of phenylglyoxal, its possible action on other membrane functions was analysed. Different parameters were investigated (Table 2). First, activities of two brush-border-membrane enzymes, alkaline phosphatase and aminopeptidase M, were shown to be unaffected by a 2 min pre-incubation with phenylglyoxal at 25°C, conditions under which phosphate transport is inhibited by 67%. However, alkaline

Fig. 5. Effect of pH on phenylglyoxal inhibition of phosphate uptake by brush-border-membrane vesicles. Membrane vesicles were pre-incubated for 2 min at 25°C with (○) or without (●) 50 mM-phenylglyoxal in 300 mM-mannitol in 10 mM-Mes (4-morpholine-ethanesulphonic acid)/Tris (pH 5.5–6.5), Tris/Hepes (pH 7.5–8.5) or Caps [3-(cyclohexylamino)-propanesulphonic acid]/KOH (pH 9.5–10.5), and uptake was measured in medium containing 100 mM-mannitol, 100 mM-NaSCN, 200 μ M-[³²P]-orthophosphate and 10 mM of the same buffer used for pre-incubation. The percentage inhibition is shown for each pH (▲).

Fig. 6. Effect of phenylglyoxal inhibition on Na⁺-dependent phosphate uptake by brush-border-membrane vesicles. Membrane vesicles were pre-incubated for 2 min at 25°C in 300 mM-mannitol/10 mM-Tris/Hepes buffer, pH 7.5, with (○) or without (●) 50 mM-phenylglyoxal. Vesicles were then diluted in the phosphate-uptake medium, and uptake was measured as described in the Materials and methods section.

Table 2. Effects of phenylglyoxal on brush-border-membrane enzymes and phosphate uptake			
Membrane vesicles were pre-incubated for 2 min at 25°C with or without 50 mM-phenylglyoxal. Enzyme activities were assayed and phosphate uptake was measured as described in the Materials and methods section. Intravesicular volume was measured by glucose uptake at steady state. Na ⁺ -H ⁺ exchange was measured at 1 mM-Na ⁺ .			
	No phenylglyoxal (control)	50 mM-Phenylglyoxal	Effect (%)
Alkaline phosphatase (μ mol of <i>p</i> -nitrophenol/min per mg of protein)	2.38 $\pm$ 0.02	2.38 $\pm$ 0.01	0
Aminopeptidase M (μ mol of <i>p</i> -nitroaniline/min per mg of protein)	2.86 $\pm$ 0.81	2.77 $\pm$ 0.57	3
Intravesicular volumes (μ l/mg of protein)	1.7 $\pm$ 0.3	1.6 $\pm$ 0.3	6
Na ⁺ -H ⁺ exchange (nmol of Na ⁺ /5s per mg of protein)	1.05 $\pm$ 0.10	0.98 $\pm$ 0.09	7
Na ⁺ -independent phosphate uptake (pmol of phosphate/20s per mg of protein)	59 $\pm$ 0.6	54 $\pm$ 7	7
Na ⁺ -dependent phosphate uptake at steady state (pmol of phosphate/60min per mg of protein)	610 $\pm$ 25	580 $\pm$ 50	5
Na ⁺ -gradient-dependent phosphate uptake (pmol of phosphate/20s per mg of protein)	2340 $\pm$ 140	784 $\pm$ 95	67

Fig. 7. *Effect of phenylglyoxal on Na⁺-dependence of phosphate uptake in brush-border-membrane vesicles* Membrane vesicles were pre-incubated for 2 min at 25°C as in Fig. 4, with (○) or without (●) 50 mM-phenylglyoxal, and phosphate uptake was measured in 100 mM-mannitol/10 mM-Tris/Hepes buffer, pH 7.5, containing the indicated amounts of NaSCN, as indicated in the Materials and methods section. Mannitol was used to maintain osmolarity. The inset represents percentage inhibition calculated from the results. Mean values $\pm$ S.D. of three experiments are given.

Fig. 8. *Eadie-Hofstee plot of the data shown in Fig. 7, with the same symbols and units*

phosphatase activity was affected by longer pre-incubation with the reagent, and a half-time of inactivation of 9 h was estimated (results not shown) as compared with 45 s for phosphate transport. Intravesicular volumes measured by glucose uptake at equilibrium were not significantly affected by phenylglyoxal treatment. The Na⁺-H⁺ antiport activity is also intact in treated vesicles. Table 2 also shows the lack of effect on Na⁺-independent phosphate uptake, measured in the presence of 100 mM-KSCN and on phosphate uptake at equilibrium after dissipation of the NaSCN gradient.

Effect of phenylglyoxal on Na⁺ transport

In order to test whether phenylglyoxal inhibition of phosphate, glucose and alanine uptake could simply be due to dissipation of Na⁺ gradient by an ionophore-like effect of phenylglyoxal, we studied Na⁺ uptake under the same conditions as phosphate uptake. Fig. 9 shows that phenylglyoxal had no effect on the time course of Na⁺ uptake either in initial rates or in steady state. Initial rates of Na⁺ uptake, measured at 5 s, were also unaffected by phenylglyoxal at different magnitudes of the Na⁺

Fig. 9. Effect of phenylglyoxal on Na^+ uptake by brush-border-membrane-vesicles

Membrane vesicles were pre-incubated as indicated in Fig. 2 legend with (○) or without (●) 50mM-phenylglyoxal, and Na^+ uptake was measured in medium containing 100mM-mannitol, 100mM- $^{22}\text{NaSCN}$ and 20mM-Tris/Hepes buffer, pH 7.5, as described in the Materials and methods section.

Fig. 10. Effect of phenylglyoxal on Na^+ uptake by brush-border-membrane vesicles in different gradient conditions

Membrane vesicles were pre-incubated as indicated in Fig. 2 legend with (○) or without (●) 50mM-phenylglyoxal, and Na^+ uptake was measured in medium containing 100mM-mannitol, 20mM-Tris/Hepes buffer, pH 7.5, and the indicated concentrations of $^{22}\text{NaSCN}$, as described in the Materials and methods section. Osmolarity was maintained with choline chloride.

Fig. 11. Effect of phenylglyoxal on Na^+ efflux from brush-border-membrane vesicles

Membrane vesicles were loaded with 100mM-mannitol, 100mM- $^{22}\text{NaSCN}$ and 20mM-Tris/Hepes buffer, pH 7.5, for 1h at 25°C. They were then incubated with (○) or without (●) 50mM-phenylglyoxal for 2min at 25°C and diluted (1:20) into 300mM-mannitol/20mM-Tris/Hepes buffer, pH 7.5. Samples were taken and filtered at indicated times, as described in the Materials and methods section.

gradient (Fig. 10). Na^+ efflux was also measured (Fig. 11) in the presence and in the absence of phenylglyoxal and was found to be unchanged, ruling out any ionophoric effect of the inhibitor.

Effect of transmembrane electrical potential on phenylglyoxal inhibition

When the transmembrane electrical potential was short-circuited by K^+ and valinomycin (Table 3), phosphate, glucose and alanine uptakes were found to be unaltered. This also indicates that the contribution of electrical potential is minimal in these conditions. The extents of the phenylglyoxal inhibition were identical in both conditions, showing that inhibition was not caused by alteration of transmembrane electrical potential.

Effect of protection of Na^+ -binding sites on phenylglyoxal inhibition of phosphate transport

In order to characterize further the site of phenylglyoxal action, attempts were made to protect the Na^+ -binding site by adding NaSCN during the pre-incubation with phenylglyoxal. Results in Table 4 show that the arginine residues modified by phenylglyoxal were not protected by Na^+ under these conditions. Amiloride and harmaline are

Table 3. *Effects of transmembrane electrical potential on phenylglyoxal inhibition of phosphate uptake by brush-border-membrane vesicles*

Membrane vesicles were equilibrated in 200mM-mannitol/50mM-KSCN/20mM-Tris/Hepes buffer, pH 7.5, for 90min at 25°C and pre-incubated with or without 50mM-phenylglyoxal for 2min. Phosphate uptake was then measured in a medium containing 100mM-NaSCN and 50mM-KSCN in 20mM-Tris/Hepes buffer, pH 7.5, and 200 μ M- $K_2H^{32}PO_4$ or 100 μ M-L-[^{14}C]alanine or 100 μ M-D-[^{14}C]glucose in the presence or in the absence of valinomycin (10 μ g/mg of protein).

	Valinomycin absent (control)			Valinomycin present		
	Uptake (pmol/20s per μ g of protein)			Uptake (pmol/20s per μ g of protein)		
	No phenylglyoxal	50mM-Phenylglyoxal	Inhibition (%)	No phenylglyoxal	50mM-Phenylglyoxal	Inhibition (%)
Phosphate	2.203 $\pm$ 0.108	0.686 $\pm$ 0.039	69	2.190 $\pm$ 0.126	0.599 $\pm$ 0.072	73
Glucose	0.419 $\pm$ 0.019	0.045 $\pm$ 0.012	89	0.433 $\pm$ 0.042	0.051 $\pm$ 0.018	88
Alanine	0.342 $\pm$ 0.049	0.044 $\pm$ 0.020	87	0.280 $\pm$ 0.089	0.025 $\pm$ 0.012	91

Table 4. *Effect of harmaline, amiloride and Na⁺ on phenylglyoxal inhibition of phosphate uptake by brush-border-membrane vesicles*

Membrane vesicles were resuspended and pre-incubated for 2min at 25°C in the media listed in the Table, with or without phenylglyoxal. When amiloride and harmaline were used, they were added 30s before addition of 50mM-phenylglyoxal. After pre-incubation, membrane vesicles were diluted 1:100 in 300mM-mannitol/20mM-Tris/Hepes buffer, pH 7.5, centrifuged and equilibrated for 5min at 25°C in the same solution used for dilution. The washing procedure was repeated two more times before phosphate transport was measured as described in the Materials and methods section.

Pre-incubation medium	Phosphate uptake (pmol/20s per μ g protein)		Inhibition (%)
	No phenylglyoxal	50mM-Phenylglyoxal	
300mM-Mannitol	2.205 $\pm$ 0.105	0.704 $\pm$ 0.087	69
300mM-Mannitol + 5mM-harmaline	1.972 $\pm$ 0.112	0.472 $\pm$ 0.093	76
300mM-Mannitol + 1mM-amiloride	1.894 $\pm$ 0.067	0.828 $\pm$ 0.043	56
150mM-KSCN	2.299 $\pm$ 0.088	0.562 $\pm$ 0.013	76
150mM-NaSCN	2.592 $\pm$ 0.229	0.702 $\pm$ 0.007	73

both reversible inhibitors of the Na⁺-H⁺ exchange in the luminal membranes, and, in addition, harmaline is a competitive inhibitor of the Na⁺-binding sites on Na⁺ symports (Aronson & Bounds, 1980). Pre-incubation with either compound affords no protection from inhibition of phosphate transport by phenylglyoxal. Na⁺ uptake at 1mM-Na⁺, which represents Na⁺-H⁺-exchange activity (Kinsella & Aronson, 1980), is not inhibited by phenylglyoxal treatment. This indicates that the modified arginine residues are not located on the Na⁺-H⁺-exchanger, as one would have expected had there been a partial protection by harmaline and amiloride. The lack of protection by harmaline also indicates that the site of action of phenylglyoxal is not an Na⁺-binding site.

Discussion

In the present study we report that Na⁺-driven uptake of phosphate, glucose and alanine by brush-border-membrane vesicles is inhibited by reagents that modify arginine. α -Dicarbonyl compounds

such as butanedione, methylglyoxal and phenylglyoxal, under the conditions used here, are specific reagents for arginine residues (Riordan, 1979). Stabilization of butanedione inhibition by borate ion (Riordan, 1979) and the pH-dependent reaction of phenylglyoxal (Takakashi, 1968) are also indications that modifications have occurred on arginine residues. Phenylglyoxal reacts considerably faster than methylglyoxal and butanedione, which might indicate a hydrophobic environment for the site modified. Vesicle integrity is preserved during the phenylglyoxal treatment, as intravesicular volume, passive fluxes and marker enzyme activities are not affected.

The magnitude of the Na⁺ gradient on the inhibition by phenylglyoxal appears to be critical only at low Na⁺ concentrations (under 30mM), where the inhibition by phenylglyoxal increases progressively to reach and maintain a 65% level. Phosphate transport at pH 7.5 is an electroneutral process, and the stoichiometry of the Na⁺/phosphate co-transport is 2:1 (Hoffman *et al.*, 1976; Cheng & Sacktor, 1981). Taking these observa-

tions into account, the inhibition of phosphate transport by phenylglyoxal does not change the affinity of the free Na^+ -binding sites implicated in phosphate transport, whereas the maximum number of Na^+ -binding sites in the membrane is decreased. This would indicate that the phenylglyoxal inhibition is not at the Na^+ -binding site. Lack of protection by Na^+ on the inhibition by phenylglyoxal confirms this conclusion; the positive charge of arginine at pH 7.5 could not be compatible with an Na^+ -binding site.

Four points indicate that the inhibition observed for phosphate, glucose and alanine cannot be due to a gross alteration of the membrane caused by a non specific action of phenylglyoxal, as follows. (1) Na^+ -independent uptake of P_i is unaffected by phenylglyoxal inhibition. (2) Na^+ uptake and efflux are independent of phenylglyoxal inhibition. (3) Intravesicular volumes are not affected by phenylglyoxal treatment. A gross modification of all membrane arginine residues would have sufficiently altered protein conformation to cause changes of intravesicular volumes. (4) Incubation of brush-border-membrane vesicles with [^{14}C]-phenylglyoxal followed by polyacrylamide-gel electrophoresis has not revealed any labelled protein (results not shown), which is expected if carriers represent only about 0.4% of total membrane proteins. This absence of labelling is a strong confirmation that phenylglyoxal is a selective modifier for highly reactive arginine. Some of the proteins of the brush-border membrane represent up to 5% of total membrane proteins; if phenylglyoxal would react with all arginine residues, structural and catalytic, those proteins would have been strongly labelled and would have shown up in our analysis.

The modified arginine residues that appeared to be implied in phosphate, glucose and alanine transport are highly reactive towards phenylglyoxal. For comparison, alkaline phosphatase, known to have an essential arginine residue at the catalytic site (Daemen & Riordan, 1974), has a half time of phenylglyoxal-induced inactivation 500 times that observed for inhibition of phosphate transport. This indicates a very special microenvironment favouring high reactivity for the arginine residues implicated in transport. In most of the enzymes studied so far, phenylglyoxal reacts only with the arginine residue located at the catalytic site, with no effect on the other residues of the enzyme (Riordan, 1979). The high reactivity of arginine at catalytic sites is the result of a lower pK_a of the guanidinium group, which itself is a result of a restricted microenvironment. The extensive loss of transport activity caused by phenylglyoxal suggests that the arginine residues that are modified are directly implicated

in Na^+ -substrate-carrier translocation. It is hardly conceivable that the modification of an arginine residue situated far from an essential site for translocation could cause sufficient changes in the transporter structure to cause complete loss of activity.

Arginine is not the only amino acid thought to be important in brush-border-membrane transport. Amino groups, tyrosine and thiol groups have been implicated in glucose translocation (Lin *et al.*, 1972; Weber & Semenza, 1983; Biber *et al.*, 1983). Some of the brush-border-membrane amino groups could be protected by the simultaneous presence of Na^+ and sugar substrates, but others could not.

In conclusion, arginine residues appear to be important in the translocation step associated with phosphate, glucose and alanine transport across the renal brush-border membrane; modification of arginine would inhibit the presumed conformational changes associated with the symporters.

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