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THE EFFECT OF pH ON α-METHYL-D-GLUCOSIDE ACCUMULATION BY RAT KIDNEY CORTEX SLICES

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SUMMARY

The kinetics of α-methyl-D-glucoside accumulation by rat kidney cortex slices under conditions of varying extracellular pH are compared with values obtained at pH 7.4. At pH below 7.4 there is a diminished initial uptake and reduced influx of the sugar which results in a decrease in the steady-state intracellular pool. This was associated with a decrease in the V of the entry process without affecting the apparent K_m of transport. At pH 8 there is no change in the rate of glucoside entry. The efflux of the glucoside, however, is impaired and the steady-state concentration gradient becomes greater than that observed at pH 7.4.

INTRODUCTION

During the course of our investigations of the characteristics of the transport of rat kidney cortex slices of α-methyl-D-glucoside, a non-metabolizable model sugar, we observed a dependence of the uptake process on the pH of the incubation media [1]. Over the pH range 6.0 to 8.4 maximum uptake during a 30-min incubation occurred between 7.4 and 8.0. Because the mechanism for sugar transport may involve the interaction of the substrate with a carrier, presumably protein in nature, it seemed that insight into the nature of the process could be obtained by a kinetic analysis of the pH effect. In enzyme–substrate interactions studies of the changes in K_m with pH have been instrumental in delineating the chemical nature of the site responsible for saturable substrate binding. We, therefore, undertook experiments to determine the kinetics of α-methyl-D-glucoside transport by rat kidney cortex under conditions of varying external pH and to define the relationship of the apparent transport K_m to pH. Our observations form the basis of this report.

METHODS

Male Sprague–Dawley rats weighing 150–200 g used in all experiments were fed a Purina Chow diet and water ad libitum until being sacrificed by decapitation.

The techniques employed for the preparation of kidney cortex slices, aerobic incubation in Krebs–Ringer bicarbonate buffer at 37 °C, calculation of intracellular and medium concentration of α-methyl-D-[U-^{14}C]glucoside concentration and the application of Michaelis–Menten kinetics to sugar transport studies have been described in detail previously [1, 2].

Results are expressed as the distribution ratio, the cpm/ml of intracellular fluid to cpm/ml medium. The distribution ratio represents a concentration gradient since α-methyl-D-glucoside is not metabolized in the tissue. Concentration of the sugar in the tissue is obtained by multiplying the distribution ratio by the medium substrate concentration. This was done to derive the velocity in concentration dependence experiments.

Conditions employed by us in transport experiments for varying medium pH have been reported previously [1, 3]. At pH above 7.4 the $NaHCO_3$ of the Krebs–Ringer buffer was replaced by 0.1 M Tris buffer at the appropriate pH. At pH 7.0, 6.5 and 6.0 Krebs–Ringer phosphate buffer was employed. Concentration of Na^+ was kept constant. Neither Tris nor phosphate affected transport since identical results to those in Krebs–Ringer bicarbonate have been obtained for glucoside with these buffers at pH 7.4 (ref. 1). The buffer pH was taken at the start and at the completion of 30-min incubations. The Tris buffer at pH 8 decreased 0.2 pH unit while the phosphate buffer at pH 6.0 rose 0.1 pH unit. Such changes in buffer pH have been reported by Kleinzeller et al. [4] who incubated rabbit kidney slices over the same pH range using Tris–*N*-tris(hydroxymethyl)methylaminoethane sulfonate (TES) buffers.

The extracellular space of the cortical slices determined with [^{14}C]inulin and total tissue water determined by the difference between wet and dry weight was 26 and 79%, respectively [5], and was unchanged by the manipulation of pH.

Efflux was measured as described previously [1] by incubating slices with 2 mM α-methyl-D-[U-^{14}C]glucoside for 60 min after which the tissue was removed, quickly rinsed in physiological saline, blotted, and transferred to flasks containing 3 ml of media. The vessels were then gassed and sealed. At 3-min intervals the flasks were opened and the medium sampled for radioactivity. At the end of 18 min the tissues were removed and the ^{14}C content of the tissue assessed. The total counts effluxed into the medium and the counts remaining in the tissue after 18 min were summed to determine the label present at the onset of the efflux phase.

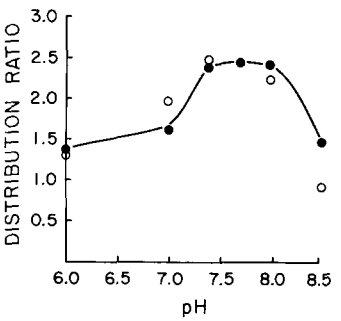
The two-compartment analysis of α-methyl-D-glucoside kinetics was performed as described by McNamara et al. [6] based on the multicompartmental analysis of steady state kinetics described for kidney slices by Rosenberg et al. [7]. All linear curves were fitted to the data with a Monroe Computer (Model 1775).

α-Methyl-D-[U-^{14}C]glucoside (73.4 Ci/mole) was obtained from Calbiochem, Los Angeles, Calif. This was found to correspond to a known standard on thin-layer chromatography [1]. Unlabeled α-methyl-D-glucoside was purchased from the Pfanstiehl Co., Waukegan, Illinois and found to be pure and free of glucose by gas–liquid chromatography [1]. [*Carboxyl*-^{14}C]Inulin was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS

Effect of pH on concentrative uptake

The uptake of α-methyl-D-glucoside by rat kidney cortical slices after a 30-min incubation at 37 °C is shown in Fig. 1. The distribution ratio is maximal between pH 7.4 and 8.0 and corroborates the data we have published previously [1]. (The distribution ratio at pH 6 and 7, 1.62 ± 0.12 and 1.36 ± 0.04, respectively, differs from that at pH 7.4, 2.38 ± 0.05, $P < 0.001$). Because Kleinzeller and associates [4] reported little or no effect of pH on the uptake of this glucoside by rabbit cortical cells incubated at 25 °C we carried out the experiment at 25 °C to see if temperature was a factor in the pH effect. As demonstrated in Fig. 1 the effect of pH is essentially the same at both 37° and 25 °C.



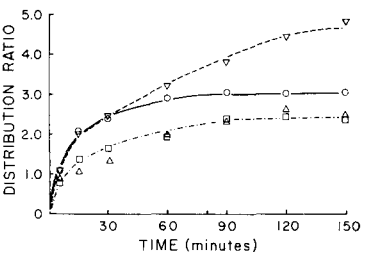


Fig. 1. Effect of pH on the uptake of α-methyl-D-glucoside by rat kidney cortex slices. Three slices, one from each of three animals, total weight 20–45 mg were incubated for 30 min in 2 ml of buffer containing 2 mM sugar and 0.1 μCi/ml of label in a Dubnoff shaker. Krebs–Ringer bicarbonate buffer was used at pH 7.4. Buffers were modified Krebs–Ringer solutions with phosphate at pH below 7.4 and Tris above 7.4. Uptake is designated as the distribution ratio, the ratio of cpm per ml intracellular fluid to cpm per ml medium. ●–●, 37 °C, each point is an average of six determinations; ○, 25 °C, each point is an average of three determinations.

Fig. 2. The influence of pH on the time course of the accumulation of α-methyl-D-glucoside by cortical slices. Conditions were described in Methods and Fig. 1. Incubation temperature was 37 °C. △–·–·–△, pH 6.0; □–·–·–□, pH 7.0; ○–○, pH 7.4; ▽- - -▽, pH 8.0. Each point is an average of from 6 to 18 determinations.

Since the accumulation of sugars (and amino acids) by kidney cortex slice is a complex process dependent on entry and exit rates, data obtained at one time point such as in Fig. 1 may not reflect the entire picture of the pH effect. Fig. 2 shows the accumulation of α-methyl-D-glucoside over a 150-min period of incubation at pH 6, 7, 7.4 and 8. Compared to pH 7.4, at pH 6 and pH 7 there is a significantly lower uptake curve throughout the period of study. At pH 7 the distribution ratio after 5 min is 0.83 ± 0.03 compared to 1.10 ± 0.03 at pH 7.4 (6 determinations, $P < 0.001$) and at 90 min is 2.38 ± 0.07, differing significantly from the 3.02 ± 0.06 at pH 7.4 ($P < 0.001$, 13 determinations).

At pH 8.0 the glucoside uptake is the same as at pH 7.4 for the first 30 min of incubation (5-min distribution ratio at pH 8 is 1.07 ± 0.03). After this time the sugar accumulation increases progressively. At 90 min of incubation the distribution

ratio at pH 8.0 is 3.80±0.22 compared to the control of 3.02±0.06, $P < 0.001$. A steady state is achieved at pH 8.0 after 120 min, the distribution ratios at 120 and 150 min, 4.44 and 4.76, respectively, not being statistically different (6 determinations).

The effect of medium pH on the velocity of uptake was studied at various α-methyl-D-glucoside concentrations and Fig. 3 is a Lineweaver–Burk double reciprocal plot of the data obtained. Lowering the pH from 7.4 to 7.0 did not significantly alter the apparent K_m (4.4 mM compared to 4.2 mM) but decreased the V from 10.9 to 6.6 mmoles/l per 30 min. Decreasing the H^+ concentration (pH 8.0) did not alter the relationship of velocity of uptake to substrate concentration, all of the points falling along the line calculated for pH 7.4.

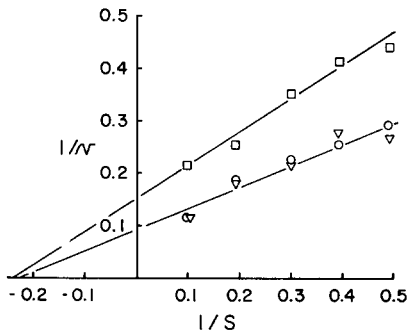


Fig. 3. Lineweaver–Burk plot of the effect of pH on α-methyl-D-glucoside uptake. v is velocity expressed as mmoles/l per 30 min and has been corrected for diffusion [19]. S is α-methyl-D-glucoside concentration in mM. ○–○, pH 7.4; each point at pH 7.4 is an average of nine determinations. The line drawn was determined to be the best fit of the data using a Monroe 1775 computer and is represented by the equation $Y = 0.416\ X + 0.092$; □–□, pH 7.0 each point representing an average of six determinations. The equation for the line at pH 7.0 is $Y^1 = 0.635\ X^1 + 0.151$; ▽–▽, pH 8.0, three determinations. The fitted line for data points at pH 8.0 was the same as that for pH 7.4.

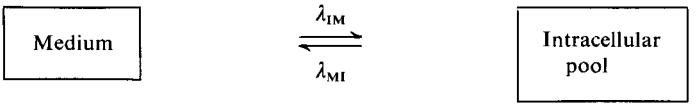
Kinetic parameters affected by pH

Since previous studies have shown that uptake curves obtained by incubating with 2 mM labeled sugar are essentially the same as those of tracer amounts of radioactive compound added after unlabeled α-methyl-D-glucoside has reached a steady state [6], the data represented in Fig. 2 can be analyzed assuming a closed two-compartment system. The calculated parameters of the two-compartment system are shown in Table I. When the medium pH is reduced from pH 7.4 to 7.0 the steady state pool is decreased from 0.318 to 0.254 μmoles with a 25% decrease in net flux. This occurs with a decrease of the influx rate constant, λ_{IM}, of 25% but with little alteration in the efflux rate constant, λ_{MI}. Raising the medium pH to 8.0 does not alter the net flux but increases the steady state pool by 54% compared to pH 7.4. The fractional rate constant for entry, λ_{IM}, is essentially unchanged but there is a 37% decrease in λ_{MI}, the efflux rate constant, from 0.0733 to 0.0465 min^{-1}. Thus, pH lower than 7.4 affects primarily the entry process while pH above 7.4 affects the exit process.

TABLE I

INFLUENCE OF EXTRACELLULAR pH ON KINETICS OF α-METHYL-D-GLUCOSIDE TRANSPORT

All calculations are based on 100 mg of tissue and an intracellular space of 53 % of wet tissue weight. The rate constants are related by the equation $M \cdot \lambda_{IM} = ICS \cdot \lambda_{MI}$ where M is the medium concentration and ICS is the intracellular pool.



pH	Medium pool size (μmoles)	Steady state distribution ratio	Steady state intracellular pool (μmole)	Fractional turnover rate (min^{-1})		Net flux (μmole/min per 100 mg wet weight)
				λ_{IM}	λ_{MI}	
7.0	4	2.4	0.254	0.00440	0.0693	0.01760
7.4	4	3.0	0.318	0.00583	0.0733	0.02332
8.0	4	4.6	0.488	0.00567	0.0465	0.02268

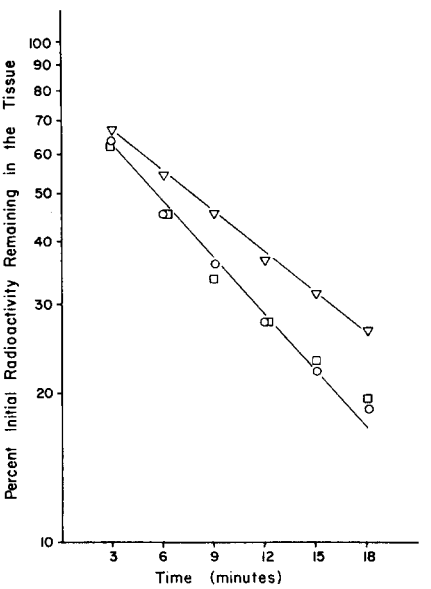


Fig. 4. Effect of pH on the efflux of α-methyl-D-glucoside from rat kidney cortex slices. Slices accumulated the radioactive glucoside for 60 min at 37 °C from medium containing 2 mM and 0.1 μCi/ml of label. As described in Methods, the slices were transferred to new buffer without sugar and the radioactivity appearing in the medium was assayed at 3-min intervals. ○–○, pH 7.4, quadruplicate determinations; □–□, pH 7.0 and ▽–▽, pH 8.0, duplicate determinations.

Observed changes in sugar efflux

Direct observations of efflux were made with slices which accumulated the sugar during incubation at pH 7.0, 7.4 and 8.0, and the results are shown in Fig. 4.

The efflux rate constant at pH 7.4 was 0.0912 min^{-1}. Data obtained at pH 7 fit along the efflux curve determined at pH 7.4 and confirm the lack of a significant effect of low pH on efflux as calculated in Table I. At pH 8.0 the efflux was slower than at pH 7.4. The rate constant calculated to be 0.0642 min^{-1} is 30% less than that at pH 7.4 and corresponds to the 37% decrease in λ_{MI} calculated by steady-state analysis (Table I) of the uptake curves shown in Fig. 2.

DISCUSSION

Our experiments indicate that altering the medium pH to values lower or higher than pH 7.4 significantly affects the transport of α-methyl-D-glucoside by rat kidney cortex slices. A kinetic analysis shows that there are entirely different effects depending on which side of pH 7.4 the change is made. At pH 7.0 influx is diminished without a change in efflux, the total flux is lower and the steady state pool size is decreased. At pH 8.0 efflux is slower without a change in influx, there is no alteration in total flux but yet a marked increase in the steady state pool size. Independent experiments of uptake and efflux confirm the kinetic analysis. The lack of insight into the effects of pH change which occurs when data obtained at a single time point are considered (as in Fig. 1) strengthens our conviction that solute transport by cells which possess entry and exit components requires time-dependent flux estimations [8]. Such estimations have recently permitted greater understanding of temperature [6], Na^+ [9], and cyclic AMP [10] effects on α-methyl-D-glucoside transport by kidney cortex slices as well as a more judicious view of amino acid transport parameters in the same tissue [7, 11–13].

The mechanism of the inhibition of sugar entry at pH 7 does not involve an alteration in the apparent K_m but a decrease in the maximum velocity of the process. This suggests that there is no change in the affinity of the saturable site(s) that may be involved in the transport mechanism. The decrease in maximum velocity could be explained by a decrease in the number or the efficiency of the saturable sites. A lack of any K_m change precluded further studies on the ionizable nature of the sugar binding site. An increase in external H^+ may cause these effects but one cannot exclude an intracellular metabolic effect of H^+ which could modify sugar influx. In this regard, Rorive et al. [14], studying the effects of pH variation (from 6.2 to 8.2) on rabbit kidney cortex slices at 25 °C observed no change in O_2 consumption, ATP levels, or osmotic properties of the cells. Kleinzeller et al. [4] under the same conditions showed that lower medium pH increases intracellular K^+ while lowering Na^+ and alkaline pH increases cellular Na^+ and decreases K^+. Since the efflux process must occur at the inner aspect of the cell membrane it may well be that such modifications of intracellular electrolytes may be responsible for the slower efflux at pH 8.0.

Although we have been able to calculate influx and efflux rate constants by analysis of a simple two-compartment system, the actual localization of sugar entry and exit to luminal and antiluminal membranes of the kidney cortical cells cannot be determined. The prevailing data suggest that sugars may enter through both the brush border and basal membrane but exit primarily at the basal membrane [15, 16]. It is thus possible that the different kinetic results of incubation of kidney cortical slices at pH 7 and pH 8 may be due to modulation of the influx process at the luminal

brush border membrane as a result of increasing H^+ and regulation of the exit process at the basal membrane as a consequence of decreasing H^+.

The results reported here with rat kidney cortex slices incubated at 37 °C differ from those reported for α-methyl-D-glucoside uptake by Kleinzeller et al. [4] using rabbit kidney cortex incubated at 25 °C. The latter experiments showed little difference in the uptake of α-methyl-D-glucoside incubated at pH 6.2, 7.2 and 8.2 for 60 min only. Incubation of rat cortical slices at 25 °C did not obliterate the pH dependence of the entry process (Fig. 1) which leads us to conclude that a species difference is responsible for the observed variation.

The most extensive examination of the effect of pH on sugar uptake by kidney cortex slices is that of Kleinzeller et al. [4] who besides the aforementioned experiments with α-methyl-D-glucoside studied the accumulation of 2-deoxy-D-glucose, 2-deoxy-D-galactose and galactose by rabbit tissue. Increasing pH from 6.2 to 8.2 produced a 3-fold increase in the steady state accumulation of 2-deoxy-D-glucose but a marked decrease of 2-deoxy-D-galactose uptake. In both cases the change in uptake was due to modification in the apparent K_m of entry. Galactose uptake was reported to be increased with pH which was in part ascribed to a decrease in efflux of galactose. The interpretation of galactose transport is complicated, however, by its metabolism [18] and, indeed, the nature of the intracellular metabolites at each pH was not identified nor was the identity of effluxed compounds determined [4]. These data strongly support the concept that the uptake of these sugars is mediated by separate processes responding differently to pH shifts.

It would seem unlikely that studies carried out with extreme variation in pH about pH 7.4 have physiological significance with regard to intact kidney glucose reabsorption. It would be a highly unusual and life-threatening situation for plasma pH in a human to reach pH 7.0. Moderate acidosis can be seen in the human disorder, renal tubule acidosis, but glycosuria is not a concomitant finding [17]. It may be, however, that the local pH of urine in the proximal convoluted tubule may be important in regulating sugar transport at the luminal membrane. Gottschalk et al. [20] have shown the pH of urine falls from 7.4 to 7.0 along the proximal tubule where glucose is reabsorbed. The bearing of our studies on this remains to be determined. These studies of the pH effect on transport systems in vitro, however, do provide information about the basic attributes of this important membrane function.

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