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THE STRUCTURAL REQUIREMENT FOR C_1–OH FOR THE ACTIVE TRANSPORT OF D-MANNOSE AND 2-DEOXY-D-HEXOSES BY RENAL TUBULAR CELLS

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SUMMARY

The transport of methyl-α-glycopyranosides of D-mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose, as well as of 1,5-anhydro-2-deoxy-D-glucitol in slices of rabbit kidney cortex was investigated.

1. In contrast to the respective actively transported hexoses, their methyl-glycopyranosides (0.1–1 mM) were not accumulated in the cells against marked concentration gradients. The entry of the glycosides into the cells was not significantly affected by 0.5 mM ouabain or absence of Na^+, and was only slightly inhibited by 0.5 mM phlorizin.

2. The glycopyranosides (5 mM) did not inhibit the active transport of the respective hexoses (1 mM).

3. 1,5-Annydro-2-deoxy-D-glucitol (0.1–1 mM) was not accumulated in the cells against its concentration gradient, and its entry was not affected by 2-deoxy-D-glucose. Conversely, the active transport of 2-deoxy-D-glucose (1 mM) was not significantly inhibited by a 5-fold molar excess of its 1-deoxy-derivative.

4. It is concluded that, as opposed to the structural requirements for the transport of D-glucose and D-galactose, C_1–OH is essential for the interaction with the carrier(s) mediating the active transport of D-mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose.

INTRODUCTION

The active accumulation by renal tubular cells of 2-deoxy-D-glucose and 2-deoxy-D-galactose differs in several respects from the active transport of some other sugars. As opposed to the system(s) involved in the active transport of saccharides such as methyl-α-D-glucopyranoside, methyl-α-D-galactopyranoside or a considerable portion of D-galactose, the active transport of both 2-deoxy-D-hexoses is: (1) Not coupled to Na^+ transport, and thus is not inhibited by the absence of Na^+, by ouabain, or by the absence of K^+ in the medium [1–3]. (2) Is relatively insensitive

to phlorizin, but is somewhat more sensitive to phloretin [4]. These results led to the suggestion [2, 4] that separate transport pathways may be operative.

It has been pointed out previously [1, 5, 6] that for the interaction of D-glucose or D-galactose with the transport carrier in renal cortical cells, a free hydroxyl on C_1 is not required. This conclusion was reached in view of the observations that: (a) The respective methyl-D-glycopyranosides are readily actively accumulated by the cells, and (b) these hexoses and their glycopyranosides mutually competed for the transport sites. It thus was of interest to investigate whether 2-deoxy-D-hexoses which are actively accumulated in the renal tubular cells by a mechanism differing from that of D-glucose or D-galactose (and their methyl-glycosides), also display a similar absence of a structural requirement of C_1-OH for their transport. In this communication data are presented showing that the methyl-α-glycopyranosides of D-mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose are not actively accumulated by renal cells, and also do not compete with the respective hexoses for the transport sites. Similarly, 1,5-anhydro-2-deoxy-D-glucitol is not actively transported, and does not compete with 2-deoxy-D-glucose for the carrier. Thus, in the absence of C_2-OH in the D-*gluco* configuration, a free C_1-OH is mandatory for the interaction between the actively transported sugar and the carrier.

MATERIALS

D-Mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose were purchased from Sigma Chemical Co., St. Louis, Mo. Methyl-α-glycopyranosides of these hexoses were prepared by the general procedure of E. Fischer, i.e. treatment of the sugars with methanolic HCl: Methyl-α-D-mannopyranoside [7], m.p. 193 °C (uncorr.); methyl-α-2-deoxy-glucopyranoside [8], m.p. 90 °C; methyl-α-2-deoxy-D-galactopyranoside [9], m.p. 112 °C.

D-[1-^{14}C]Mannose and 2-deoxy-D-[1-^{14}C]glucose were obtained from New England Nuclear Corp., Boston, Mass.

1,5-Anhydro-2-deoxy-D-glucitol, and also this compound ^{3}H-labeled at C_1 and C_2, were a gift from Dr J. R. Griggs, of Smith, Kline and French Laboratories, Philadelphia, Pa. Both compounds were chromatographically pure.

Preparation of labeled sugars and methyl-α-glycopyranosides

2-Deoxy-D-[^{3}H]galactose was prepared by catalytic exchange at the Radiochemical Centre, Amersham, Buckinghamshire, England. The crude product (50 mCi) was then purified by repeated paper chromatography employing two solvent systems, i.e.: (a) ethylacetate–acetic acid–water containing 2 % (w/v) phenylboronic acid (9:2:2, v/v/v); followed by: (b) *n*-butanol–ethanol–water (104:66:30, v/v/v). The radioactivity was localized in a sharp peak. The sugar was eluted from the paper with water and the solution was lyophilized. The sugar was stored at − 20 °C in an aqueous solution containing 20 % (v/v) ethanol. Radiochemical yield of the pure substance: 54 %; specific activity: 124 Ci/mole. A small batch of 2-deoxy-D-[1,2-$^3H_2$]-galactose was also prepared by the addition of diluted ^3H_2O to D-galactal [10]. The specific activity of the chromatographically purified preparation was 49 μCi/mmole. The radiochemically determined R_F values of this and the former preparation in both above solvent systems were identical and coincided with those of authentic

2-deoxy-D-galactose.

The ^{14}C-labeled methyl-α-glycopyranosides of D-mannose and 2-deoxy-D-glucose were prepared starting with 100 μCi each of the respective 1-^{14}C compounds. 500 μCi of 2-deoxy-D-[^{3}H]galactose were used for the preparation of the corresponding methyl-glycoside. The reactions were carried out on a microscale under conditions employed for the non-labeled compounds by placing the dry-labeled sugar plus 10 mg of unlabeled sugar and the corresponding amount were maintained in an oil bath at 90 °C for the required time period. The neutralized methanolic solutions were taken to dryness and the sugars were chromatographically purified. Sharp peaks, with radiochromatographically determined R_F values identical with those found for the authentic cold glycosides were then eluted from the paper with water and the extracts were taken to dryness by lyophilization. The radiochemical yield was around 50 %.

METHODS

Experiments described below were carried out employing slices of rabbit kidney cortex. Unless otherwise stated, experimental conditions and analytical procedures used were those presented in detail in previous communications (see e.g. ref. 5). In most experiments, the incubation media were of the Krebs–Ringer type [5] containing 128.5 mM Na^+ as the bulk cation (Na^+-saline); Li^+-saline denotes a medium in which Na^+ was equivalently replaced by Li^+.

The methyl-glycosides tested in the experiments given below appeared to be non-metabolizable by renal tissue: The found values of total tissue saccharides (after extraction of the tissue with 5% (w/v) ice-cold trichloroacetic acid, or boiling water) were within the limits of experimental error identical with those found after treatment of the neutral aqueous extract with $ZnSO_4$+$Ba(OH)_2$ (see ref. 11 for a preliminary statement of the analytical procedure); thus, no evidence for phosphorylation of the methyl-glycosides by the tissue was obtained. On the other hand, when testing the effect of these glycosides on the transport of the respective hexoses, values for both the total (i.e. free plus phosphorylated) and free sugars (after precipitation of sugar phosphates by the $ZnSO_4$+$Ba(OH)_2$ procedure) are given.

The results are expressed as the accumulation ratio S_i/S_0, where S_i is the computed intracellular concentration of the saccharide after correction for the sugar in the extracellular, i.e. inulin, space), and S_0 is the final concentration of the sugar in the incubation medium. In experiments where the effect of the glycosides on the transport of the metabolizable hexoses is described, the results are presented in μmole tissue sugar per g tissue wet wt. Mean values, ±S.E. are given.

RESULTS AND DISCUSSION

Table I presents data on the uptake of 1 mM methyl-α-D-glycopyranosides of D-mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose (denoted as Me-Man, Me-dGlc and Me-dGal, respectively) by slices of rabbit kidney cortex. It will be noted that in the controls no significant cellular accumulation of the glycosides against their concentration gradient took place. In experiments not given here in detail, no uphill transport of the tested substrates was observed when their external concentration was lowered to 0.1 mM. The absence of observable active transport of these

TABLE I

TRANSPORT OF METHYL-α-GLUCOPYRANOSIDES BY SLICES OF RABBIT KIDNEY CORTEX

Slices were first incubated 45 min aerobically (O_2) at 25 °C in the respective media, then were incubated under identical conditions for 60 min in the presence of 1 mM of the respective methyl-α-glycopyranosides without (control) or with inhibitors. The results are the means of 4–5 determinations ±S.E. For abbreviations see text.

Medium	Inhibitor	Me-α-Man S_i/S_o	Me-α-dGlc S_i/S_o	Me-α-dGal S_i/S_o
Na	None (control)	1.10±0.05	1.03±0.04	1.08±0.02
Na	Phlorizin, 0.5 mM	0.89±0.03	0.94±0.01	0.84±0.05
Na	Ouabain, 0.5 mM	0.82±0.02	0.99±0.02	0.91±0.05
Li	None	0.94±0.02	1.02±0.03	1.02±0.03

glycosides was not due to a rather slow transport process; time-curve experiments showed that the cellular space was filled with the respective saccharide within 30 min, and their apparent cellular concentration did not increase further during subsequent incubation for additional 90 min. Data given in Table I also show that experimental conditions such as the presence of 0.5 mM ouabain or the absence of external Na^+ had no significant effect on the cellular level of the tested glycosides. Phlorizin (0.5 mM) was slightly inhibitory.

D-Mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose are actively transported by renal tubular cells (refs 1 and 5 and data given below). Results presented in Table I rather surprisingly indicate that elimination of the free C_1-OH by conversion of the respective hexoses to their methyl-α-glycopyranosides abolishes the active accumulation of the sugars. The entry of the glycosides of both 2-deoxy-hexoses into renal cells in accordance with their concentration gradient was within the limits of experimental error independent of external Na^+ and was ouabain-insensitive; such result was not surprising in view of the known absence of Na^+ requirement for the active transport of the respective hexoses [1, 2]. The Na^+ dependence of D-mannose transport has been previously described [5]. Phlorizin is a not particularly effective inhibitor of the transport of 2-deoxy-hexoses, in contrast to its effectiveness as an inhibitor of the transport of saccharides such as D-galactose or methyl-α-D-glucopyranoside [1, 4].

Next, the affinity of the methyl-glycosides for the carrier(s) mediating the transport of D-mannose, 2-deoxy-D-glucose and 2-deoxy-D-galactose was tested by investigating whether the glycosides exerted an inhibitory effect on the active transport of the respective hexoses. The results of this study are presented in Table II.

It will be noted that all three hexoses were readily taken up by the tissue and a considerable (intracellular) phosphorylation took place (see also ref. 12 for transport and phosphorylation of 2-deoxy-D-glucose in renal cells). The computed S_i/S_o ratio of the free 2-deoxy-hexoses was significantly higher than 1.0 (S_i/S_o: 2-deoxy-D-glucose: 3.02±0.18; 2-deoxy-D-galactose: 3.51±0.26), evidence for their active accumulation against a concentration gradient. Using the improved analytical procedure for the determination of free and phosphorylated sugars, no direct evidence for an uphill accumulation of the free D-mannose was found under the given experi-

TABLE II

ACCUMULATION OF D-MANNOSE, 2-DEOXY-D-GLUCOSE AND 2-DEOXY-D-GALACTOSE IN SLICES OF RABBIT KIDNEY CORTEX: THE EFFECT OF THE RESPECTIVE METHYL-α-D-GLYCOPYRANOSIDES THEREON

Tissue was first preincubated 45 min aerobically (O_2) at 25 °C in standard Na^+ medium, and subsequently incubated under identical conditions in media containing 1 mM of the respective sugars without (control) and with 5 mM of the methyl-glycosides derived from these hexoses. The results are the means of 4–6 determinations, ±S.E.

Sugar	Control		Methyl-glycoside present	
	Tissue sugar (μmoles/g)		Tissue sugar (μmoles/g)	
	Total	Free	Total	Free
D-Mannose	1.49±0.01	0.74±0.02	1.38±0.05	0.67±0.05
2-Deoxy-D-glucose	7.82±0.28	1.38±0.06	6.41±0.12	1.23±0.03
2-Deoxy-D-galactose	4.21±0.15	1.70±0.10	4.66±0.06	1.48±0.07

mental conditions ($S_i/S_0 = 1.02 \pm 0.03$). However, it has to be borne in mind that the (intracellular) phosphorylation of the sugar does depress the steady-state level of free mannose.

The presence of the glycosides (5 mM) did not significantly affect the uptake and accumulation of the corresponding hexoses. It is concluded that the elimination of the free C_1-OH by conversion to the corresponding methyl-α-glucopyranosides abolishes the affinity of the respective sugars to the carrier(s) mediating their transport. This result thus supports the conclusion based on a comparison of the transport of the investigated hexoses and their glycosides (Table I).

As an argument against such conclusion it might be pointed out that the introduction of a bulky methyl group at C_1 can be inhibitory for steric or hydrophobic reasons in addition to the change of C_1-OH. If this were so, the following predictions should be borne out experimentally: (1) The methyl-glycosides of other actively transported sugars would be expected to show the same behaviour as those tested above. This is not the case. Both α- and β-methyl-D-glucopyranosides are transported into renal cells against high concentration gradients [1,2]. Also, under identical experimental conditions methyl-β-D-galactopyranoside accumulates in renal cells against the same concentration gradient as free galactose, and both monosaccharides appear to share the same carrier (ref. 6, and unpublished data). Thus, hexoses with C_2-OH in the D-*gluco* configuration (i.e. D-glucose and D-galactose) lack a structural requirement of C_1-OH for their active transport. (2) The transport properties of sugars in which the hydroxyl on C_1 was eliminated by a less bulky constituent should be comparable to those of the parent hexoses. This point was examined using 1,5-anhydro-2-deoxy-D-glucitol (i.e. 1,2-deoxy-D-glucose). The substitution of C_1-OH in 2-deoxy-D-glucose by the less bulky C_1-H abolished active transport: The substance readily entered the cellular space and reached within 45 min a steady tissue level, the accumulation ratio S_i/S_0 not exceeding the value of 1.3 (5 experiments). As shown in Table III, Expt. 1, the S_i/S_0 was not affected by varying the concentration of 1,2-deoxy-D-glucose (0.1–1 mM). The entry of the substance (0.1 mM) across the cellular membrane was inhibited by cold but was not affected by 1 mM 2-deoxy-D-

TABLE III

THE TRANSPORT OF 1,5-ANHYDRO-2-DEOXY-D-GLUCITOL BY SLICES OF RABBIT KIDNEY CORTEX

Experimental conditions were those given in the legend to Tables I and II. Abbreviations: 1,5-anhydro 2-deoxy-D-glucitol: 1,2-dGlc; 2-deoxy-D-glucose: 2-dGlc.

Expt No.	1,2-dGlc (mM)	Addition	Tissue, 1,2-dGlc S_i/S_o
1	0.1	none	1.17±0.02
	0.5	none	1.05±0.03
	1.0	none	1.25±0.04
	2.0	none	1.06±0.01
2	0.1	none (control)	1.30±0.03
	0.1	2-dGlc, 0.1 mM	1.15±0.03
	0.1	2-dGlc, 0.5 mM	1.28±0.04
	0.1	2-dGlc, 1.0 mM	1.28±0.06

TABLE IV

EFFECT OF 1,5-ANHYDRO-2-DEOXY-D-GLUCITOL ON THE ACCUMULATION OF 2-DEOXY-D-GLUCOSE IN SLICES OF RABBIT KIDNEY CORTEX

Experimental conditions were those given in the legend to Table II. 2-deoxy-D-glucose: 1 mM; 1,5-anhydro-2-deoxy-D-glucitol: 5 mM. The results are the means ±S.E., 6 analyses.

Addition	Tissue 2-deoxy-D-glucose (μmoles/g)	
	Total	Free
None (control)	7.82±0.28	1.38±0.06
1,5-Anhydro-2-deoxy-D-glucitol	6.78±0.28	1.26±0.05

glucose (Table III, Expt 2), 2-deoxy-D-galactose or other sugars. Also, 0.1–1 mM phlorizin, phloretin, ouabain and *N*-ethylmaleimide were ineffective as inhibitors, in contrast to their effects on the transport of 2-deoxy-D-glucose. Expt 2 in Table III shows the lack of effect of 2-deoxy-D-glucose on the entry of 1,2-deoxy-D-glucose. These results thus provide further evidence for the conclusion that sugars lacking C_2-OH in the D-*gluco* configuration require a free C_1-OH for their interaction with the transport carrier(s).

The above results are also taken as evidence for the view [4–6] that several carriers with defined (possibly overlapping) specificities are involved in the active transport of sugars in renal cells. The present data do not allow conclusions to be drawn as to whether one or several carriers are involved in the transport of D-mannose and both 2-deoxy-D-hexoses. Data on the mutual competition of these sugars for transport sites will be reported elsewhere.

Evidence that in renal cells, D-mannose is transported by a pathway differing from that of D-glucose, has been also obtained by other authors by two experimental approaches: Silverman et al. [13] investigated in vivo the transport specificity of sugars at the luminal face of renal cells and inferred from their data the existence of two

pathways: (a) A pathway shared by D-glucose, 2-deoxy-D-glucose and D-galactose; (b) a D-mannose-transport system. Using preparations of isolated brush borders of renal tubular cells it was found that 2-deoxy-D-galactose [14] and 2-deoxy-D-glucose [15] shared with D-mannose the property of competing with phlorizin for the low-affinity binding sites; in addition, 2-deoxy-D-glucose also appeared to some extent to interact with the high-affinity binding sites for phlorizin. These data are compatible with results reported above that in renal cells, sugars lacking C_2-OH in the D-*gluco* configuration differ in their transport properties from other hexoses. The observation that 2-deoxy-D-glucose interacts with two affinity sites in brush borders may be related to the observation of Silverman et al. [13] that D-mannose is considerably more effective as an inhibitor of the reabsorption of 2-deoxy-D-glucose than of D-glucose. Thus, data obtained by studies in vivo and with brush-border preparations support the view that several carriers with differing (overlapping) specificities participate in the active sugar transport in renal cells.

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