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# STUDIES ON THE TRANSPORT OF ALIPHATIC GLUCOSIDES BY HAMSTER SMALL INTESTINE IN VITRO*

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## SUMMARY

It has been found (1) that glucosides with a long alkyl chain (2–18 carbon atoms) as the aglycone can be transported by carrier-mediated processes in the hamster small intestine in vitro, (2) that these glucosides interact with the glucose carrier, and (3) that they compete with glucose and analogs for the binding to the carrier. There are $Na^+$- and phlorizin-insensitive components of uptake for the long chain alkyl glucosides which suggest additional interactions or uptake processes.

## INTRODUCTION

We have synthesized a number of new D-glucosides and tested them as substrates for the $Na^+$-dependent glucose transport system of hamster small intestine, in vitro. These glucosides have alkyl aglycones of various chain lengths terminated by a methyl group, a hydroxyl group or a second glucose unit in glycosidic linkage. Our findings are reported below.

## MATERIALS AND METHODS

Some of the compounds used in the study were obtained from commercial sources: β-methyl D-glucoside and D-galactose from Sigma Chemical Co.; phlorizin from Calbiochem; D-[U-$^{14}$C]galactose with a specific activity of 42.4 Ci/mol from New England Nuclear Corp.; β-methyl D-[U-$^{14}$C]glucoside with a specific activity of 52.5 Ci/mol was obtained from Calbiochem.

D-Glucosides other than those above were prepared in our laboratory by the modified Koenigs-Knorr method [1, 2] which yields the β-isomer exclusively. The

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physical and chemical properties of these compounds will be reported elsewhere. Some preliminary experiments not reported herein were performed with a sample of *n*-decyl α-D-glucoside generously given by Atlas Chemical Industries.

*Incubation and analytical methods.* Small intestines from male hamsters, 9–10 weeks old, with free access to food and water were used in making everted segments to restrict access of test compounds to the mucosal surface. These segments were made by everting the intestine onto a length of polyethylene tubing (PE280), double-tying short lengths to the tubing and then cutting between ties [3]. About 0.3 g total tissue weight of the everted preparations were incubated with shaking in 10.0 ml of modified Krebs-phosphate [4] buffer contained in 50 ml Erlenmeyer flasks gassed with $O_2$. For solubilization of the long chain glucosides, addition of 4 mM sodium taurocholate was found to be necessary. Hence, it was added routinely in all tests including controls. At the end of the incubation period, the everted preparations were collected, blotted and the tissue removed from the polyethylene tubing and weighed. The tissues were transferred to 4 ml of 80 % ethanol, homogenized and centrifuged. The supernatant liquids were evaporated to dryness, heated with 1 M HCl at 100 °C for 1 h in sealed tubes and neutralized with NaOH. The amount of glucose liberated by hydrolysis was determined by the glucose oxidase method [5, 6], and was taken as a measure of glucoside transport. This method gives 90–95 % recoveries of glucose from the compounds used.

Radioactivity was measured by a Beckman liquid scintillation counter, using the medium prepared as described by Patterson and Green [7]. Results are expressed as rate of entry in μmol of sugar accumulated per ml of tissue water in a specified time, assuming a water content of 80 % of the wet tissue weight [8].

## RESULTS AND DISCUSSION

### *Studies with alkyl glycosides*

Eight β-glucosides with alkyl aglycone chain lengths in the range of 2–18 carbon atoms were synthesized in all. However, initial experiments were done with decyl and dodecyl β-glucosides in order to test whether such compounds may serve as substrates for transport.

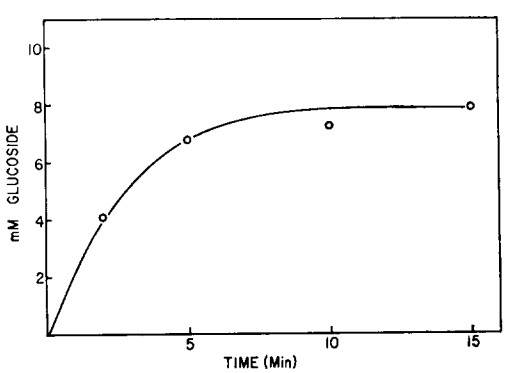


Fig. 1. Uptake of decyl glucoside as a function of time. Incubation was in 10.0 ml of buffer with 1 mM decyl glucoside.

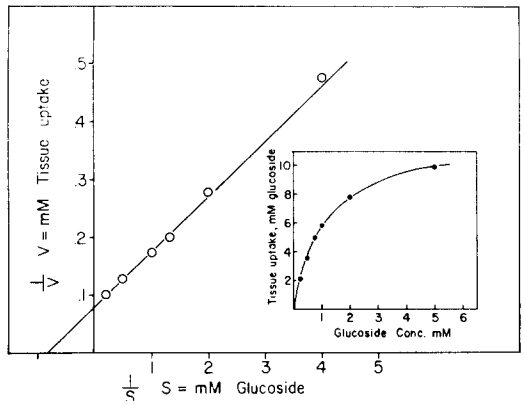


Fig. 2. Lineweaver-Burk plot for decyl glucoside uptake. Incubation was in 10.0 ml of buffer for 5 min. The inset shows the effect of increasing concentration of decyl glucoside on its uptake.

With decyl glucoside, maximal uptake required about 5 min at which time an apparent tissue/medium ratio of about 7.0 was established (Fig. 1). Such a ratio would ordinarily be taken to indicate that transport had occurred against a concentration gradient. The process of uptake is strongly inhibited (57 %) by 2,4-dinitrophenol at 1 mM.

With graded concentrations of decyl glucoside, uptake increased with concentration and tended towards saturation (inset, Fig. 2). A Lineweaver-Burk plot (Fig. 2) of the same data indicates that the uptake process is saturable as is characteristic of carrier-mediated transport. It also indicates the existence of a single quantitatively important process (or multiple processes indistinguishable from one another in $K_m$ and $V$).

Tests of competition for transport with the actively transported glucose analog, $\beta$-methyl glucoside, were made with dodecyl glucoside. As seen in Fig. 3, dodecyl

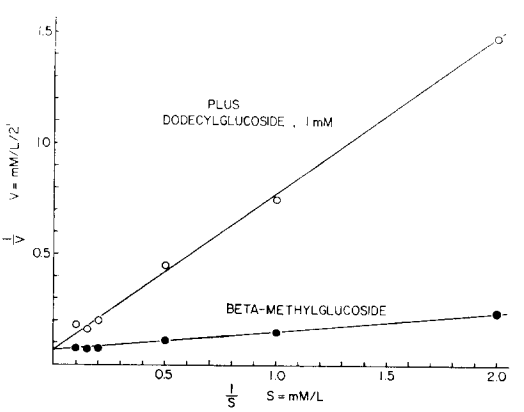


Fig. 3. Lineweaver-Burk plot for $\beta$-methyl glucoside uptake in the presence and absence of dodecyl glucoside. Incubations were in 10.0 ml of buffer for 2 min. Dodecyl glucoside was at 1 mM. Corrections for extracellular $\beta$-methyl glucoside were made from corresponding mannitol controls.

TABLE I

COMPARISON OF UPTAKE OF ALKYL GLUCOSIDES

All the substrates were at a concentration of 1 mM and of the $\beta$ configuration. Period of incubation, 15 min. Values represent mean $\pm$ S.D. for at least four experiments.

| Alkyl glucoside | Glucoside in tissue water (mM) |
|---|---|
| Methyl glucoside | 30.6±1.3 |
| Ethyl glucoside | 29.6±1.1 |
| Butyl glucoside | 8.2±1.2 |
| Hexyl glucoside | 2.9±0.6 |
| Octyl glucoside | 2.9±0.3 |
| Decyl glucoside | 10.5±2.3 |
| Dodecyl glucoside | 7.9±3.2 |
| Tetradecyl glucoside | 5.7±1.8 |
| Octadecyl glucoside | 3.7±0.77 |

glucoside strongly inhibited the uptake of $\beta$-methyl glucoside with competitive kinetics. The $K_i$ is 0.2 mM.

Tests were also done to see whether the alkyl glucosides were hydrolysed by the intestinal tissue. There was no hydrolysis of the glucosides either by intact tissue or by intestinal homogenates.

*Effect of aglycone chain length on uptake*

Landau et al. [9], tested methyl, ethyl, isopropyl and butyl $\beta$-glucosides as substrates for transport and found that methyl, ethyl and isopropyl glucosides were well accumulated while butyl glucoside was transported to a lesser extent. Our results (Table I) confirm these earlier studies. Our results also show that with further increase in chain length, uptake continued to diminish to octyl glucoside, increased at decyl

TABLE II

EFFECT OF THE OMISSION OF $Na^+$ ON UPTAKE OF GLUCOSIDES

Choline chloride was used to substitute for NaCl in Krebs-Ringer phosphate buffer. Potassium taurocholate was used to solubilize the glucosides. Period of incubation, 15 min. Values represent mean $\pm$S.D.

| | Glucoside in tissue water (mM) | Inhibition (%) |
|---|---|---|
| Decyl glucoside | | |
| in $Na^+$ buffer | 9.1±1.3 | |
| in choline$^+$ buffer | 6.6±0.6 | 27.5 |
| Dodecyl glucoside | | |
| in $Na^+$ buffer | 8.0±1.3 | – |
| in choline$^+$ buffer | 4.7±1.2 | 41.2 |
| Tetradecyl glucoside | | |
| in $Na^+$ buffer | 5.7±1.6 | |
| in choline$^+$ buffer | 4.0±0.7 | 30.0 |

glucoside and then diminished progressively thereafter. A reason for the reversal of trend between octyl and decyl and the decrease after decyl is not available. However, the former may possibly be due to the solubility characteristics of the glucosides. Up to octyl glucoside, the compounds are grossly water soluble; from decyl on, they are grossly not water soluble.

*Effect of phlorizin*

Phlorizin is a potent competitive inhibitor of the glucose transport system [10]. Hence, the effect of phlorizin on glucoside transport was studied to test for the involvement of the glucose carrier. Phlorizin totally inhibited the uptake of glucosides with chain lengths from 1 to 8 carbon atoms in the aglycone. This was taken to indicate that the total uptake of these glucosides was by means of the glucose carrier. However, with increased aglycone chain length, phlorizin inhibition dropped to the range of 40–50 % and was 56±2, 43±2 and 41±4 % for decyl, dodecyl and tetradecyl glucosides, respectively. The phlorizin-sensitive component of uptake may be taken as a measure of the contribution of the glucose carrier. However, the phlorizin-insensitive component may measure either (i) non-specific binding attributed to the insertion of the distal end of the fatty chain into the lipoidal matrix of the membrane, or (ii) uptake by another route. We have recently prepared acyl derivatives of glucose which provide similar alkyl chain lengths at carbon 1 (Ramaswamy, K., Bhattacharyya, B. R. and Crane, R. K., unpublished). Binding of these compounds is negligible. They do attach to the binding site of the glucose carrier and are effective non-penetrating competitive inhibitors of the uptake of glucose analogs. Hence, it may be inferred that the phlorizin-insensitive component of long chain glucoside transport is not related to non-specific binding of the long alkyl chain to the membrane surface and is more likely related to the glucose moiety.

*Effect of the omission of $Na^+$*

As is well known [11, 12], $Na^+$ is required for the intestinal transport of glucose in vitro. Hence, studies were done to observe any effect of the omission of $Na^+$ on uptake of glucosides. There was almost no uptake for glucosides with chain lengths 1–8. With increase in alkyl chain length, there was a reduction of uptake by the omission of $Na^+$ for decyl, dodecyl and tetradecyl glucosides but it was in the range of only 30–40 %, even less than the range of inhibition by addition of phlorizin

TABLE III

COMPARISON OF UPTAKE OF ALKYL AND HYDROXYLATED ALKYL GLUCOSIDES

All of the substrates were used at a concentration of 1 mM. Period of incubation, 15 min. Values are mean ±S.D.

| Glucoside | Glucoside in tissue water (mM) |
|---|---|
| Butyl glucoside | 7.7±0.8 |
| 4-Hydroxybutyl glucoside | 3.0±0.4 |
| Hexyl glucoside | 1.6±0.1 |
| 6-Hydroxyhexyl glucoside | 0.8±0.2 |

TABLE IV

EFFECT OF DODECYL DIGLUCOSIDE ON UPTAKE OF GLUCOSE AND GALACTOSE

Period of incubation, 15 min; values represent average values of two experiments.

| | Glucose or galactose in tissue water (mM) | Inhibition (%) |
|---|---|---|
| 1 mM glucose | 14.5 | – |
| + 1 mM dodecyl diglucoside | 4.7 | 68 |
| 1 mM galactose | 7.0 | – |
| + 1 mM dodecyl diglucoside | 2.0 | 72 |

(Table II). The meaning either of the level of reduction or of the difference from that of phlorizin inhibition is not obvious.

*Studies with hydroxylated alkyl glucosides*

4-Hydroxybutyl and 6-hydroxyexyl glucosides were tested as substrates for uptake. These compounds differ from the glucosides described above in that they have a hydroxyl group terminating the aglycone chain. It can be seen from Table III that these glucosides with the more hydrophilic side chain are transported to a lesser extent than the parent alkyl glucosides.

*Effects of dodecyl diglucoside on uptake of substrates of the glucose transport system*

Dodecyl diglucoside has two glucose moieties separated by 12-$CH_2$-groups and both ends of the molecule are potential substrates of the glucose transport system. Dodecyl diglucoside was not detectably taken up by in vitro hamster small intestinal preparations.

However, it had a highly inhibitory effect on the transport of both glucose and galactose (Table IV), indicating that it interacts well with the binding site of the glucose carrier.

ACKNOWLEDGEMENT

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