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Interactions of alkylglucosides with the renal sodium/D-glucose cotransporter

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Abstract

To study the nature of the glucose binding pocket of the renal Na^+/D-glucose cotransporter, we have evaluated the inhibitory potency of various alkylglucosides (AG) on sodium-dependent D-glucose uptake into hog kidney brush border membrane vesicles (BBMV). Inhibition at 0.1 mM AG level was found to be strongly dependent on the anomeric configuration, on the length and on the flexibility of the side chain. β-n-AG inhibited transport significantly more effectively compared to the corresponding α-anomer (n-octylglucoside: α-anomer 15%, β-anomer 84%) and AG with an unsaturated n-alkenyl side chain were significantly less effective inhibitors than the corresponding saturated compound (cis/trans 3-n-β-hexenylglucoside 53% and 32%, β-n-hexylglucoside 76%). A series of β-n-AG increasing in side chain length from 1 to 13 carbon atoms revealed a global maximum in the inhibition pattern when β-AG with side chains ranging from 8 to 11 carbon atoms were used, thus β-methylglucoside inhibited glucose transport by 13%, β-n-nonylglucoside by 92%, and β-n-tridecylglucoside showed no effect. Kinetic analysis of inhibition by β-n-octylglucoside revealed a fully competitive type of inhibition with an apparent K_i of 10 ± 2 μM. n-Octylglucoside at 0.1 mM did not inhibit sodium-dependent L-alanine uptake; similarly, n-octylmannoside at 0.1 mM level did not affect D-glucose uptake. These results suggest that the inhibition of sodium-dependent D-glucose uptake was, at least in the concentration range tested (up to 0.1 mM), not due to a detergent effect of AG, but due to interaction with the carrier. Optimum interaction requires a β-anomer with a glycosidic bond that places the alkyl chain into an equatorial position with regard to the D-glucose molecule and the two main determinants of the sugar recognition site C2 and C3. In addition, the alkyl chain has to be highly flexible. The alkyl chains thus apparently interact with hydrophobic sites at the carrier in a slightly coiled conformation, thereby AG with a chain length up to 6 carbon atoms interact only with one hydrophobic site, AG with higher chain length probably with two sites.

Keywords: Sodium/D-glucose cotransport; Brush border membrane; Alkylglucoside; Inhibitor; Glucose binding pocket; (Kidney)

1. Introduction

Several members of the Na^+/D-glucose cotransporter (SGLT) family have been identified recently by cloning experiments: the high affinity Na^+/D-glucose cotransporter from rabbit small intestine [1] and kidney [2], from human [3], hog [4] and rat kidney [5] and the low affinity Na^+/D-glucose cotransporter from human kidney [6,7]. Analysis of the deduced amino acid sequences according to the hydrophobic moment plot method [8] showed the Na^+/D-glucose cotransporter most likely to be a membrane protein with 12 membrane spanning α-helical domains. In addition, mutagenesis data identified amino acids critically important for the interaction with D-glucose and sodium [9]. Despite this information the knowledge about the topology and the assembly of different functional domains of the transporter is still very limited.

The investigation of the functional and kinetic properties of sodium-dependent D-glucose cotransport by measuring the uptake of radiolabeled substrates into isolated plasma membranes [10] obtained from hog renal cortex revealed a low affinity for D-glucose, $K_m = 1.3$ mM, and a high sensitivity for the naturally occurring β-glucoside phlorizin, $K_i = 1.2$ μM [11]. The about 1000-fold increased affinity for phlorizin compared to D-glucose can only be explained by the presence of multiple binding sites on the transporter, one being identical with the glucose binding site. The high affinity inhibitor phlorizin seems to bind to the substrate recognition site with the D-glucose moiety and to hydrophobic sites on the transporter with the β-glucoside side chain [12]. Thus the common motif of

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high affinity inhibitors is their capability of accommodating these multiple sites.

Beside phlorizin, which exhibits an aglucon moiety of a rather complicated chemical structure, there are a few reports of simple AG employed as inhibitors for the Na^+/D-glucose cotransport. AG are substrates of sodium-dependent D-glucose transport into hamster small intestine [13] and inhibitors of sodium-dependent D-glucose uptake into rabbit, rat and human small intestine brush border membrane vesicles [14].

In order to explore the spacing between multiple sites on the transporter, we have systematically investigated the inhibitory potency of AG on sodium-dependent D-glucose uptake into BBMV obtained from hog renal cortex. Taking the simple chemical structure of AG into account, a series of homologs can be easily synthesized and evaluated as inhibitors. In the present study we attempt to demonstrate that despite their detergent potential, AG can be used as high affinity inhibitors for the Na^+/D-glucose cotransporter at low concentrations and that they are valuable tools for the investigation of the assembly of different sites on the transporter molecule in its membrane embedded state.

2. Materials and methods

2.1. Materials

All chemicals used in this study were of the highest purity available. Radiolabeled substrates D-[6-^{3}H]glucose (1 TBq/mmol) and L-[2,3-^{3}H]alanine (2.18 TBq/mmol) were obtained from Amersham-Buchler (Braunschweig, Germany). The β-AG of phenyl, methyl and n-β-pentyl to n-β-dodecyl and α-phenyl-, n-α-octyl-, and n-α-decylglucoside were products of Sigma (Deisenhofen, Germany). α-n-Octylmannoside, β-n-tridecylglucoside and *cis*- and *trans*-n-3-hexenyl-β-glucoside were synthesized in our laboratory as described below.

2.2. Alkylglucoside synthesis

AG were synthesized by glycosylation of the corresponding alkanol with α-acetobromoglucose as described by Weber and Benning [15]. Synthesized AG and intermediates were purified according to the 'flash' method [16] on Silica Gel 32-63 mesh, 60A (E. Merck, Darmstadt, Germany) using the solvent systems light petroleum/ethyl acetate 3:1 for acetylated and ethyl acetate/methanol/water 27:3:1 for deacetylated compounds. Final products were homogenous on thin-layer chromatography plates (Silica Gel, 60A, E. Merck, Darmstadt, Germany) developed in the above indicated solvent systems. Identity and purity was further established by ^{1}H (500 MHz) and ^{13}C (125.8 MHz) NMR spectroscopy on a Bruker AM 500 in $CDCl_3$ (acetylated compounds) or CD_3OD solution (deacetylated compounds) with tetramethylsilane as a standard. n-Octyl-α-D-mannoside was synthesized by glycosylation of n-octanol with β-acetobromomannose as described above. β-Acetobromomannose was prepared from D-mannose by subsequent reaction with sodium acetate in acetic anhydride and hydrobromic acid in acetic acid according to the method of Rosevear et al. [17].

2.3. Isolation of renal brush border membranes

Hog kidneys were obtained in a local slaughterhouse immediately after killing of the animals. Brush border membrane vesicles (BBMV) were isolated from hog kidney cortex by a calcium precipitation method described by Vannier et al. [18] and modified in this laboratory [11]. Final BBMV were suspended at a concentration of 20 mg protein/ml in mannitol Hepes Tris (MHT) buffer (100 mmol/l mannitol, 20 mmol/l Hepes-Tris, pH 7.4). The purity of the preparation was routinely determined by assaying the marker enzyme alkaline phosphatase (E.C. 3.1.3.1.) which was enriched 9- to 12-fold. BBMV were stored at −70°C until use and diluted to a concentration of 10 mg protein/ml with MHT buffer for transport studies.

2.4. Transport measurements

Uptake of radiolabeled substrates was determined at 22°C with the rapid filtration technique as described initially by Hopfer et al. [19]. The uptake was initiated by adding 20 μl BBMV suspension (200 μg protein) to 50 μl incubation media to give 100 mmol/l mannitol, 20 mmol/l Hepes-Tris (pH 7.4), 100 mmol/l NaSCN or KSCN, 0.1 mmol/l substrate (4.17 μCi D-[^{3}H]glucose or L-[2,3-^{3}H]alanine) and the indicated inhibitor concentrations. The incubation was terminated after 5 s by adding 1 ml ice-cold stop solution (100 mmol/l NaCl, 100 mmol/l mannitol, 20 mmol/l Hepes-Tris (pH 7.4) and 0.2 mmol/l phlorizin) followed by rapid filtration of the suspension through prewetted 0.45 μm nitrocellulose filters. After rinsing with 3.5 ml ice-cold stop solution the wet filters were dissolved in 7 ml scintillation fluid and counted for radioactivity in a liquid scintillation counter. 'Equilibrium' uptakes were measured under the same conditions after 90 min incubation.

Substrate uptakes presented are mean values ± S.E. from at least three independent experiments, each performed in duplicate or triplicate. For statistical comparisons, Student's *t*-test was used. Data for the sodium-dependent D-glucose uptake are corrected for the sodium-independent uptake, measured in the presence of a potassium gradient. Sodium-independent uptake ranged from 4 to 6% of the total substrate uptake.

2.5. Protein determinations

Protein was determined according to the method of Lowry et al. [20] using bovine serum albumin as standard.

3. Results

To explore the structural requirements of glucoside side chains for optimal interaction with hydrophobic sites on the transporter several AG varying in their aglucon moiety were evaluated as inhibitors of sodium-dependent D-glucose uptake into renal BBMV. AG differing in length of the alkyl side chain, in the configuration of attachment to C-1 of D-glucose (axial or equatorial) and alkenylglucosides with a double bond in the side chain, which provides a certain limit to possible conformations, were investigated.

3.1. The effect of alkyl side chain length

The effect of increasing the length of the n-alkyl side chain is shown in Fig. 1. Whereas very short and very long side chains showed only a moderate inhibition, n-alkyl substituents ranging from 8 to 11 carbon atoms were found to be most effective. Maximal inhibition was achieved with n-nonyl-β-D-glucoside, which inhibited Na^+/D-glucose cotransport by 92% at 0.1 mM level. A local maximum in the inhibition pattern was observed when n-hexyl-β-D-glucoside was used as inhibitor.

A possible explanation for the surprisingly low inhibitory potency of AG with very long alkyl side chains (> 11 carbon atoms) could be an interaction of the AG with the lipid phase of the BBMV. The extended hydrophobic parts of these molecules could lead to a partition of AG into the lipids thereby reducing the free inhibitor concentration in the incubation media. As a consequence a lower inhibition per mol of AG would be observed. The adsorption of AG to the membranes should be directly related to the total BBMV surface employed for a single experiment. Thus the inhibition observed should be inversely related to the protein concentration. To estimate this effect the inhibition by β-AG was measured under conditions, in which the total membrane protein (vesicle surface) employed for a single experiment was successively decreased (Fig. 2). A significant dependence of the inhibitory effect on the amount of membrane protein could only be detected at a chain length above 10 carbon atoms. In experiments with AG with shorter side chains no effect was observed.

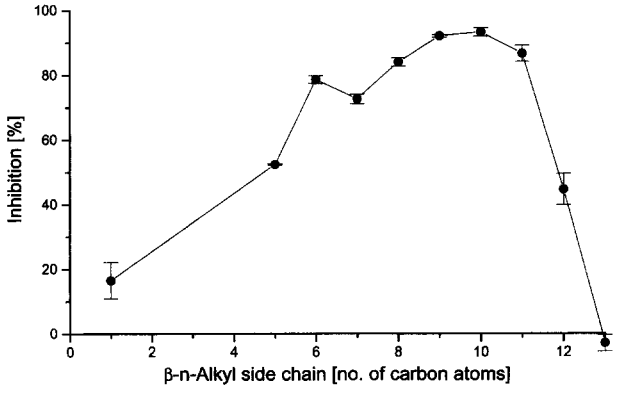

Fig. 1. Inhibitory effect of n-alkyl-β-D-glucosides (0.1 mM) increasing in length of the n-alkyl side chain from 1 to 13 carbon atoms on initial (5 s) 0.1 mM D-glucose uptake into porcine renal BBMV, mean values ± S.E. ($n = 3$).

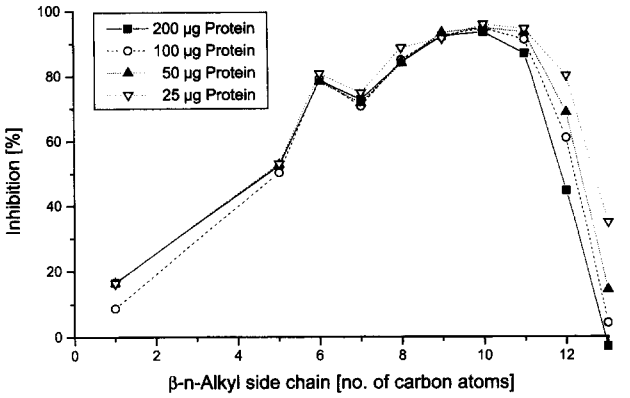

Fig. 2. Effect of protein concentration on the inhibitory effect of n-alkyl-β-D-glucosides (0.1 mM) increasing in length of the n-alkyl side chain from 1 to 13 carbon atoms on initial (5 s) 0.1 mM D-glucose uptake into porcine renal BBMV. In this experiment the ratio between the total amount of inhibitor in the incubation media and membrane protein (vesicle surface) was altered by decreasing the amount of membrane protein employed for a single experiment: 200 µg (squares), 100 µg (circles), 50 µg (up triangles) and 25 µg (down triangles) membrane protein. A representative experiment is shown, for the sake of clarity error bars are omitted, the standard deviations in this experiment were less than 5%.

3.2. The effect of altering anomeric configuration

Measuring the inhibitory effect of β-AG on 5 s sodium-dependent D-glucose uptake into BBMV compared to their α-anomers (Table 1) revealed a strong dependence on the stereochemical orientation of the substituent at C-1 of D-glucose. Inhibition effected by equatorially oriented n-alkyl side chains was significantly higher than by the axially oriented α-analogs. The β-configuration of the attachment of alkyl side chains to C-1 of D-glucose is

Table 1
Inhibition of sodium-dependent D-glucose uptake into porcine renal BBMV by α-glucosides compared to their β-anomers

Glucoside (0.1 mM)	Anomer	Inhibition [% control]	*P*-Value α / β-anomer
Phenylglucoside	α	4 ± 3	
	β	35 ± 3	0.003
Octylglucoside	α	13 ± 5	
	β	84 ± 1	0.0002
Decylglucoside	α	39 ± 6	
	β	92 ± 1	0.001

Uptake was measured for 5 s, concentration of D-glucose and the glucosides was 0.1 mM.
Mean values ± S.E. ($n = 3$). The inhibitory effect of the α-anomers is significantly lower as indicated by the *P*-values.

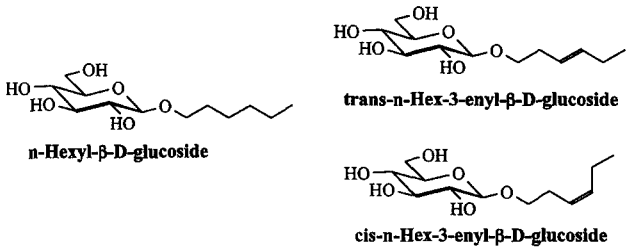

Fig. 3. Chemical structures of n-hexyl-β-D-glucoside and unsaturated n-hexenyl analogs.

Table 2
Inhibition of sodium-dependent D-glucose uptake into porcine renal BBMV by hexyl-β-D-glucoside compared to the unsaturated *cis*- and *trans*-3-hexenyl analogs

Glucoside (0.1 mM)	Inhibition [% control]
n-Hexyl-β-D-glucoside	76 ± 1
trans-n-3-Hexenyl-β-glucoside	32 ± 2
cis-n-3-Hexenyl-β-glucoside	53 ± 1

Uptake was measured for 5 s, concentration of D-glucose and the glucosides was 0.1 mM.
Mean values ± S.E. ($n = 3$). Inhibition in the presence of the two unsaturated compounds is significantly lower than in the presence of the saturated compound (*trans*: $P = 0.003$, *cis*: $P = 0.0004$) and the *cis* unsaturated compound is a significantly better inhibitor than the *trans*-isomer ($P = 0.01$).

obviously favourable for an optimal interaction with the transporter.

3.3. The effect of alkyl side chain flexibility

To study the effect of side chain flexibility, the unsaturated compounds *trans*- and *cis*-3-hexenyl-β-D-glucoside were synthesized; the chemical structures are shown in Fig. 3. Inhibition of D-glucose uptake into BBMV by both unsaturated sugars was significantly lower than by the corresponding saturated compound hexyl-β-D-glucoside, moreover the *cis*-3-hexenyl-β-D-glucoside was a significantly more effective inhibitor than the *trans*-isomer (Table 2).

3.4. The effect of altering the sugar moiety

It is well known that the Na^+/D-glucose cotransporter is very sensitive to stereochemical alteration of the D-glucose C-2, thus D-mannose for example is no substrate for the transporter [21]. To evaluate the contribution of the sugar moiety of the AG for the inhibition, the inhibitory effect of n-octylglucoside and n-octylmannoside on D-glucose uptake was compared. As shown in Fig. 4A, octylmannoside at 0.1 mM level did not significantly interfere with the transport. This experiment again indicates that the effects of AG on D-glucose transport are not due to a solubilization of BBMV, since the two octyl sugars share

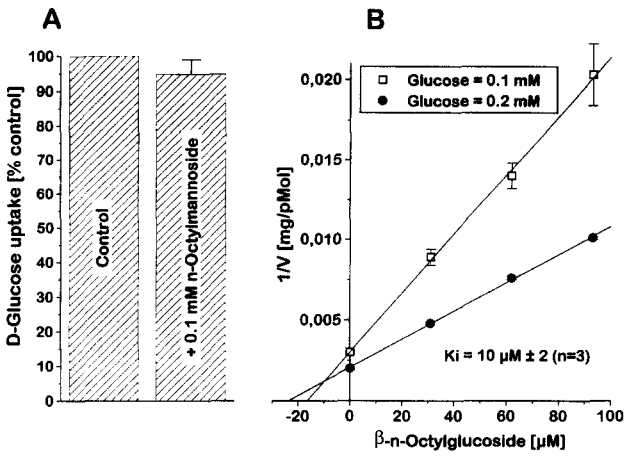

Fig. 4. (A) Effect of n-octyl-α-D-mannoside (0.1 mM) on 5 s 0.1 mM D-glucose uptake into porcine renal BBMV, mean values ± S.E. ($n = 3$). (B) Inhibitory effect of 0, 30, 60 and 90 µM n-octyl-β-D-glucoside on 5 sec D-glucose uptake into porcine renal BBMV measured at two substrate concentrations, 0.1 mM (squares) and 0.2 mM (circles) D-glucose, according to the method of Dixon. A representative experiment is shown. From three such experiments a K_i of 10 ± 2 µM was calculated.

the same non-ionic detergent potential with an identical critical micelle concentration of 30 mM. More importantly, however, the results suggest that the sugar moiety positions the alkyl chains to be able to exert their action. The importance of the interaction of the AG with the sugar binding site is also evident from the Dixon plot shown in Fig. 4B. In the concentration range tested the inhibition of D-glucose uptake by β-octyl-D-glucoside exhibits a fully competitive inhibition with regard to D-glucose.

3.5. Effects of alkylglucosides on other BBMV properties

At high concentrations AG are effective non-ionic detergents, thus the inhibition of glucose uptake observed in our studies might have been due to solubilization of membrane vesicles. Therefore the effect of representative AG on equilibrium D-glucose uptake was investigated (Table 3). At 0.1 mM the D-glucose uptake even after 90 min of

Table 3
Effect of representative alkylglucosides on equilibrium D-glucose uptake into porcine renal BBMV

Glucoside (0.1 mM)	Gradient	90 min uptake [pmol/mg]
Control	Na	203 ± 25
Control	K	180 ± 21
Methyl-β-D-glucoside	Na	185 ± 12
n-Nonyl-β-D-glucoside	Na	196 ± 16
n-Tridecyl-β-D-glucoside	Na	194 ± 27

Uptake was measured for 90 min, concentration of D-glucose and the glucosides was 0.1 mM. The equilibrium is indicated by the same D-glucose uptake rates in the presence of a sodium or a potassium gradient across the membrane vesicles in the control experiments.
Mean values ± S.E. ($n = 3$).

Table 4
Effect of representative alkylglucosides on sodium-dependent and sodium-independent L-alanine uptake into porcine renal BBMV

Glucoside (0.1 mM)	Na independent [pmol/mg]	Na dependent [pmol/mg]
Control	70±4	84±9
Methyl-β-D-glucoside	63±5	97±6
n-Nonyl-β-D-glucoside	66±5	102±4
n-Tridecyl-β-D-glucoside	67±10	116±16

Uptake was measured for 5 s, concentration of L-alanine and the glucosides was 0.1 mM.
Mean values ± S.E. ($n = 3$).

incubation was the same in the absence and presence of AG. This indicated no alteration of the intravesicular space. Similarly, sodium-independent as well as sodium-dependent L-alanine uptake was not inhibited by AG, the sodium-dependent L-alanine transporter showed even a slight although not significant increase in transport activity (Table 4). These data suggest that an alteration of general BBMV properties, such as vesicle size or membrane permeability, due to a detergent action of 0.1 mM AG can be excluded.

4. Discussion

4.1. Unspecific effects of AG

The amphiphilic character of AG with a hydrophilic sugar moiety and a hydrophobic glucoside side chain makes them effective non-ionic detergents. The distribution of these properties along the molecule also makes them potent inhibitors for the renal Na^+/D-glucose cotransporter by simultaneous interactions with the glucose recognition site and hydrophobic domains at the transporter molecule. Since solubilization of membrane proteins by AG is commonly achieved at high concentrations (n-octyl-β-D-glucoside for example has a critical micelle concentration of 30 mM), we have performed our experiments at low inhibitor concentrations of maximal 0.1 mM. To further exclude a detergent action we have limited our investigations to AG with n-alkyl side chains no longer than 13 carbon atoms and have used short incubation times (5 s) for the measurement of substrate uptakes. Within these limits no evidence for a vesicle disruption or an alteration of general BBMV properties due to a detergent action of AG has been found. In the presence of AG the L-alanine transport system present in BBMV was not affected and the equilibrium D-glucose uptake into BBMV indicated no alterations in intravesicular space. The inhibitory effect of AG with side chains longer than 10 carbon atoms is dependent on the amount of membrane protein (vesicle surface) used for a single experiment, which could be explained by a partial adsorption of the inhibitor to lipids of the vesicle surface mediated via the hydrophobic tail. However, AG with side chains up to 10 carbon atoms did not show this effect.

4.2. Effects of AG on the D-glucose transporter

Ramaswamy et al. [13] tested a series of β-n-AG with increasing length of the side chain as substrates for sodium-dependent translocation in the hamster small intestinal epithelium in vitro. Methyl and ethylglucoside were well translocated while n-butylglucoside was transported to a lesser extent and transport continued to diminish with increasing length of the AG side chain. An apparent increase of translocation activity of AG with very long alkyl chains ($\geq$ 10 carbon atoms) can be considered as an artefact due to an increased solubility of these compounds in the tissue. In our experiments also a significant binding of AG to the membranes was observed for chain lengths > 10 carbons. This indicates that translocation of AG by the Na^+/D-glucose cotransporter is limited by the size of the glucoside side group, thus too bulky side groups at C-1 prevent translocation. This observation is also supported by further work of Ramaswamy et al. [22] in which they demonstrated that 1-*O*-alkylglucosides are translocated up to a chain length of 4 carbon atoms, but that 1-*O*-acylglucosides with an additional carbonyl function at C-1 of the side chain do not undergo translocation, although being inhibitors for the transporter.

This previous work suggests that in our inhibitor studies also AG longer than n-butylglucoside do not compete with D-glucose for the translocation steps across the membrane. D-Glucose uptake into BBMV is more likely inhibited by binding of AG to the outwardly facing D-glucose binding site of the carrier with high affinity and thereby blocking D-glucose transport. This mode of AG interaction parallels the inhibitory effect of the well established inhibitor phlorizin on sodium-dependent D-glucose transport.

As shown in kinetic analysis according to the method of Dixon, n-octylglucoside is a fully competitive inhibitor of renal Na^+/D-glucose cotransport with an apparent K_i of 10 ± 2 μM with regard to D-glucose, which at first glance is surprising. Octylglucoside presumably binds not only to the D-glucose recognition site of the transporter molecule but interacts with hydrophobic sites as well and for such inhibitors interacting with multiple binding sites a mixed type of inhibition should be expected. This contradiction can be explained by assuming a two-step process for AG binding to the transporter. In the first, most important step the D-glucose moiety is recognized by the transporter and in a second step the AG side chain is positioned for the interaction with hydrophobic sites of the carrier. The predominant feature in this two-step mechanism is the binding of the D-glucose moiety to the substrate binding site which explains the fully competitive inhibition kinetic found. This assumption is also supported by the observation that octylmannoside lacks any inhibitory potential on Na^+/D-glucose cotransport, since it has a sugar moiety unable to

bind to the receptor. The intensity of interaction of AG with hydrophobic sites of the transporter molecule after binding of the D-glucose moiety was found to be strongly dependent on the properties of the side group and its structural relationship with regard to the plane of the pyranose ring of D-glucose, namely the anomeric configuration, length and flexibility of the alkyl chain were limiting factors for the inhibitory potential.

β-AG with an equatorially oriented substituent at C-1 of D-glucose were far more effective inhibitors of Na^+/D-glucose cotransport compared to the corresponding compounds with an axially oriented side group. Side groups oriented perpendicularly to the plane of the D-glucose pyranose ring in the α-anomers seem either to interfere with proper binding of the sugar moiety to the D-glucose binding site or this stereochemical arrangement interferes with access of the alkyl groups to hydrophobic transporter sites, thereby decreasing their affinity. Beside steric constrains due to bulky substituents, it may also be possible that there is an H-bond to the oxygen at C-1 in the β-position, which cannot be established with the axial oxygen of the α-anomers. These findings are also consistent with earlier observations that both β-methyl- and β-phenylglucoside are more potent inhibitors for sodium-coupled phlorizin binding to rat kidney BBMV [23] and that β-2-naphtylglucoside is the preferred substrate for sodium dependent intestinal absorption in rat [24] compared to the respective α-AG.

The degree of D-glucose uptake inhibition observed in the current study by different β-AG was dependent on the length of the alkyl side chain, thus β-AG with very short (< 8 carbon atoms) or very long side chains (> 11 carbon atoms) showed only a moderate inhibition. Whereas the low inhibition of β-AG with short alkyl side chains can be explained by the lack of access to hydrophobic transporter sites due to the limited size, one has to consider more than one effect for the low inhibition of β-AG with long alkyl side chains. Most important is the decrease of free inhibitor concentration in the incubation media due to micelle formation and adsorption of inhibitor at the vesicle surface. The critical micelle concentration for β-n-tridecylglucoside can be estimated to be 0.1 mM [25] and is only for this compound in the same concentration range where the inhibitor studies were performed (0.1 mM). Thus the decrease of the free inhibitor concentration in the incubation media due to micelle formation could be possible to a very low extent only for the highest homolog tested. The critical micelle concentration for all the other compounds is higher than 0.1 mM (β-n-dodecylglucoside 0.2 mM, β-n-undecylglucoside 0.7 mM, β-n-decylglucoside 2 mM, β-n-nonylglucoside 8 mM, etc. [25]) and a micelle formation can be excluded. For the compounds with alkyl groups longer than 10 carbon atoms, a partial adsorption at the lipids of the vesicle surface has also to be considered, as demonstrated by the protein dependence of the inhibitory effect. Whether this is the only effect responsible for the dramatic decrease of inhibitor potential from β-n-undecylglucoside to β-n-tridecylglucoside is not quite clear. In addition, it might be possible that very long alkyl side chains assuming a randomly coiled conformation are too bulky to fit into the binding pocket of the transporter. The size of the side group, which supports an optimal interaction of both D-glucose moiety and side chain with the different functional domains of the carrier, is a length from 8 to 11 carbon atoms, since these compounds were the most effective inhibitors tested. A possible explanation for the observed local maximum in the inhibition pattern (see Fig. 1) could be an interaction of the alkyl side chain with two distinct hydrophobic sites of the transporter. The one next to the glucose binding site exhibits optimal interaction with a n-hexyl residue. A further elongation of the side chain leads to a transient net loss of affinity until an optimal interaction with the two hydrophobic sites is achieved.

Since it is difficult to predict the conformational state of AG side chains while interacting with the transporter molecule, we have tried to approach this question by investigating the inhibitory potential of AG analogs with a double bond in the alkyl side chain. The side chains of the two unsaturated hexylglucoside analogs, *trans*- and *cis*-n-hex-3-enyl-β-D-glucoside, are limited in their possible conformations, because there is no rotation around the side chain bond between C-3 and C-4 (see Fig. 3). Whereas the *trans*-isomer exhibits a conformation of the side chain with a more straight character, the *cis*-isomer prefers a conformation which is more coiled. The two unsaturated compounds were significantly less effective inhibitors of Na^+/D-glucose cotransport than the hexylglucoside, indicating the importance of side chain flexibility for optimal interaction with the hydrophobic sites on the transporter. Moreover the unsaturated *cis*-isomer was a significantly more effective inhibitor than the *trans*-isomer. These data suggest that a coiled conformation of the alkyl side chain favors the access to hydrophobic sites on the transporter.

Computer supported conformational analysis using force field methods revealed that in their most likely conformers the aglucone parts of β-AG and phlorizin seem to point to different directions when bound to the glucose binding site of the transporter (S. Wielert, unpublished results). Thus it is possible that these two types of inhibitors gain their additional binding to the transporter by Van der Waal's interaction of their non-sugar moieties with completely different hydrophobic sites of the transporter, in view of the uncertainties of such modelling studies, however, direct labelling studies are necessary to clarify this question.

Our present study demonstrates that alkylglucosides are remarkable inhibitors for the renal Na^+/D-glucose cotransporter as long as a few structural requirements are met. The β-configuration, the flexibility and the choice of an appropriate length of the alkyl side chain, ranging from 8 to 11 carbon atoms, are necessary to obtain an optimal interaction. Compared to the classical inhibitor phlorizin

AG exhibit a relatively simple chemical structure, which makes them interesting tools to study the assembly of different functional domains on the Na^+/D-glucose cotransporter. Moreover, AG provide an ideal starting material for chemical modifications to obtain suitable photolabile affinity labels for the transporter, a project presently pursued in our laboratory.

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