ALKYL GLYCOSIDES AS INHIBITORS FOR THE RENAL SODIUM-D-GLUCOSE COTRANSPORT SYSTEM IN THE LITTLE SKATE (RAJA ERINACEA)

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Phlorizin is a well-known high affinity inhibitor of the sodium-D-glucose cotransport system. Its high affinity can be explained by the presence of multiple binding sites for this glycoside on the transporter, one being identical with the D-glucose binding site [Frasch et al., Pflügers Arch. 320:265-284, 1970]. In order to be able to identify the molecular nature of this binding site and to compare its organization in various species we have started to construct photolabile affinity labels which should interact with high affinity with the transporter. Since phlorizin, an aryl-glycoside has a rather complicated chemical structure we turned to simpler alkyl glycosides and investigated their inhibitory potency on sodium-D-glucose cotransport.

Brush border membrane vesicles were isolated from the kidney of male and female little skate (Raja erinacea) by a differential calcium precipitation method described earlier [Kinne-Saffran et al., Bull. MDIBL 24:61-63, 1984]. The obtained membrane fraction was about 20-fold enriched in the brush border membrane marker enzyme alkaline phosphatase and insignificantly contaminated with basal-lateral plasma membranes or intracellular membranes. Kinetic data were derived from Dixon plots by measuring the initial sodium-dependent D-glucose uptake (5 sec) into brush border membrane vesicles at substrate concentrations of 0.05, 0.10, and 0.20 mM in the presence of varying inhibitor concentrations using the rapid filtration technique [Hopfer et al., J. Biol. Chem. 248:25-32, 1973]. Experiments were performed in triplicate; n represents the number of membrane preparations usually derived from 2 to 4 skates.

Table 1

Inhibitor	Ki	n
n-hexyl-ß-D-glucopyranoside	5 µM	2
n-octyl-ß-D-glucopyranoside	12 µM	2
n-dodecyl-ß-D-glucopyranoside	>> 160 µM	3
n-octyl-ß-D-thio-glucopyranoside	12 µM	2
n-octyl-α-D-glucopyranoside	260 µM	3
phlorizin	2.5 µM	3

Mean values derived from n experiments.

The results obtained in these studies are compiled in Table 1. Comparison of the alkyl glycosides tested in the ß-series reveals a strong dependence of inhibition on chain length of the aglycon moiety. Affinity decreases with increasing chain length (n-hexyl-ß-D-glucopyranoside, K_i = 5 µM, n-octyl-ß-D-glucopyranoside, K_i = 12 µM, n-dodecyl-ß-D-glucopyranoside, K_i >> 160 µM).

This phenomenon can be explained by two factors which counteract each other: On the one hand, with increasing chain length of the alkyl moiety the ligand becomes more hydrophobic and provides a stronger interaction with a putative hydrophobic domain close to the substrate binding site. On the other hand, assuming a random coiled conformation of the alkyl side chain, the aglycon moiety of the higher alkyl glycosides becomes more bulky, which prevents proper fit into the binding pocket. An optimum seems to be reached at a side chain length of about 6 carbons, thus n-hexyl-ß-D-glucopyranoside shows an affinity comparable to phlorizin (K_i = 2.5 μM). Of minor importance is the size of the atom employed for aglyconic linkage. When the anomeric oxygen in n-octyl-ß-D-glucopyranoside is replaced by sulphur, which is 1.4 times more bulky with regard to its Van der Waal's radius, as in n-octyl-ß-D-thioglucopyranoside, no alteration in affinity can be observed. For both compounds a K_i of 12 μM was obtained.

A most interesting feature for binding requirements provides the comparison of n-octyl-ß-D-glucopyranoside with its α-anomer. These compounds are isomers and differ only in configuration at the anomeric center, C1 of D-glucose. A striking decrease in affinity is observed when the stereochemical orientation is changed from the equatorial (K_i = 12 μM) to an axial position (K_i = 260 μM). Obviously an α-alkylglucoside with an axially oriented alkyl side chain seems to exhibit a conformation less suitable for a proper binding to the protein.

Alkyl glycosides at high concentrations are commonly used as detergents, thus inhibition of glucose uptake may be due to solubilization of membrane vesicles. This possibility seems to be excluded since glucose uptake into brush border membrane vesicles measured at equilibrium in the absence and in the presence of 0.1 mM n-hexyl-ß-D-glucopyranoside, the most effective alkyl glycoside tested, was identical indicating no alteration in intravesicular space.

Our present study demonstrates that high affinity probes for the renal sodium-D-glucose cotransport system can be obtained by attachment of an aliphatic chain to the anomeric center of glucopyranose as long as a few structural requirements are taken into account. Most important is the length of the alkyl side chain, an optimum seems to be reached in the case of a n-hexyl residue, and an equatorial orientation for the anomeric linkage, which can involve either oxygen or sulphur. Thus, alkyl-glycosides seem to be a most useful starting material to obtain suitable photolabile affinity labels.

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