Biochimica et Biophysica Acta 903 (1987) 273–276
Elsevier

BBA 73682

1-*O*-*n*-Octyl-β-D-glucopyranoside as a competitive inhibitor of Na^+-dependent D-glucose cotransporter in the small intestine brush-border membrane

Maria Teresa Vincenzini, Teresa Iantomasi, Maria Stio, Cristina Treves, Fabio Favilli and Paolo Vanni

Institute of Biochemistry, University of Florence, Viale Morgagni, 50, 50134 Florence (Italy)

(Received 9 March 1987)

Key words: Octylglucopyranoside; Octyl glucoside; Cotransporter; Glucose uptake; (Intestinal vesicle)

1-*O*-*n*-Octyl-β-D-glucopyranoside is a competitive inhibitor of the Na^+-dependent D-glucose uptake into rabbit, rat and human intestinal brush-border membrane vesicles. The lack of effect on the equilibrium uptake demonstrates that the detergent does not act by rupturing the vesicles; no membrane leakiness was apparent at the concentrations of octylglucopyranoside used, since D-glucose uptake is not inhibited even in the absence of the Na^+ gradient (in K^+ solution). There is a competitive interaction between octylglucopyranoside and D-glucose, as shown by Dixon and by Hunter and Down plots. The selectivity of the detergent effect is confirmed by its modest influence on amino acid uptake.

'Natural' and synthetic detergents are widely used for solubilizing and manipulating artificial and natural membranes and for the reconstitution of transmembrane transport systems, such as the Na^+/D-glucose cotransporter [1–4]. These studies present some difficulties in that the cotransporter in the brush-border intestinal membrane occurs at very low concentrations (0.1–0.4% of the intrinsic membrane proteins [5,6]) and it becomes highly unstable following solubilization by detergents [7].

This study deals with the inhibitory action of a synthetic nonionic detergent, namely 1-*O*-*n*-octyl-β-D-glucopyranoside (octylglucopyranoside), a structural derivative from D-glucose, on the Na^+-dependent D-glucose uptake into rabbit, rat and human intestinal brush-border membrane vesicles.

We further studied the detergent's action on both the Na^+-independent D-glucose transport and the diffusional flux of L-glucose.

To determine whether octylglucopyranoside is a specific inhibitor of the Na^+-dependent D-glucose cotransporter, its effect on the Na^+-dependent transport of several amino acids, i.e. aspartic acid, lysine and phenylalanine, was also investigated.

We then explored the detergent's inhibition through both kinetic studies and experiments on mutual competition with phlorizin.

Preparation of brush-border membrane vesicles. Frozen rabbit small intestine, fresh rat small intestine and macroscopically normal pieces of human jejunum or ileum obtained during surgery were used. Intestinal brush-border vesicles were prepared by the calcium method, as first described by Schmitz et al. [8] and modified by Kessler et al. [9].

The protein concentration was about 10–20

Abbreviations: octylglucopyranoside 1-*O*-*n*-octyl-β-D-glucopyranoside; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

Correspondence: P. Vanni, Institute of Biochemistry, University of Florence, Viale Morgagni, 50 , 50134 Florence, Italy.

mg/ml, as determined by Bradford's method [10].

Transport measurements. The uptake of radiolabeled substrates into intestinal brush-border membrane vesicles was determined by the Millipore filtration technique as described by Hopfer et al. [11]. All transport studies were done at room temperature. For the inhibition and kinetic experiments detergent or phlorizin was added to the 'uptake buffer' (100 mM mannitol, 10 mM Hepes-Tris (pH 7.1)) containing vesicles and radiolabeled compounds just before the onset of the uptake reaction. After suitable incubation times (from 5 s to 60 min) transport was terminated by the addition of 3 ml ice-cold 'stop solution' (150 mM NaCl buffered with 1 mM Hepes-Tris (pH 7.1)). Other experimental conditions are described in Table I and the figure legends.

All experiments were repeated at least three times, each experiment always being triplicated.

Inhibition of D-glucose and amino acids transport by octylglucopyranoside. The time course of Na^+-dependent D-glucose uptake into brush-border membrane vesicles of rabbit small intestine, with and without 0.5 mM octylglucopyranoside, is presented in Fig.1. Equilibrium was reached at 60 min and the relative values were approximately the same. The overshoot was markedly lower ($\simeq$ 80%) with 0.5 mM octylglucopyranoside than without it. To determine whether this inhibition was selective with respect to the Na^+-dependent, but not the Na^+-independent component, we studied the effect of 1 mM octylglucopyranoside both on the D-glucose facilitated transport with a K^+ extravesicular gradient and on the L-glucose diffusional flux (Fig. 2). Since these uptake curves were not modified by octylglucopyranoside, a non-specific action at the membrane level can be excluded; at concentrations of 0.5 and 1 mM

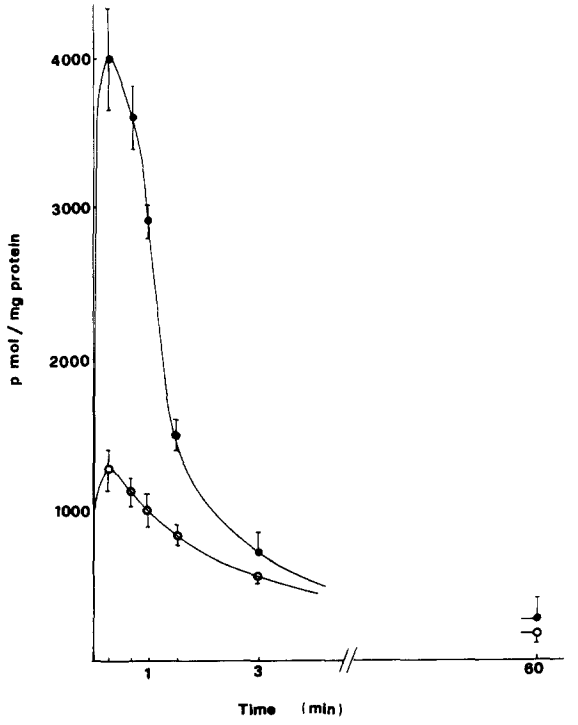

Fig. 1. Time course of Na^+-dependent D-glucose uptake into brush-border membrane vesicles. The transport was carried out at room temperature with membrane vesicles (20 μl) in 'uptake buffer' containing 110 μM D-[^{14}C]glucose, an initial NaSCN gradient (100 mM out, 0 in) in the absence (●) and in presence of 0.5 mM octylglucopyranoside (○).

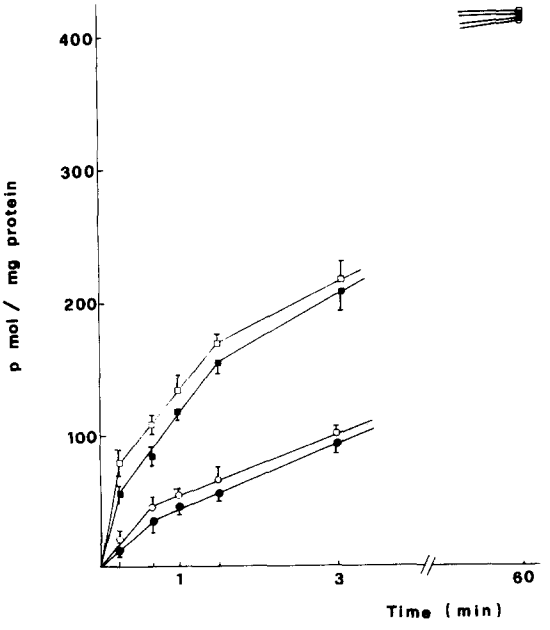

Fig. 2. Time course of Na^+ independent D-glucose uptake and diffusional flux of L-glucose into brush-border membrane vesicles. The transport was carried out at room temperature with membrane vesicles 20 μl in 'uptake buffer' containing 110 μM D-[^{14}C]glucose, an initial KSCN gradient (100 mM out, 0 in) in the absence (■) and in presence of 1 mM octylglucopyranoside (□) or 110 μM L-[^{14}C]glucose, an initial NaSCN gradient (100 mM out, 0 in) (●) and in presence of 1 mM octylglucopyranoside (○).

octylglucopyranoside no leakiness was apparent. Furthermore, we demonstrated that the inhibition by octylglucopyranoside was time-independent and reversible; in fact, when the vesicles preincubated with octylglucopyranoside were washed with 'uptake buffer', the inhibition was completely removed. As shown in Table I, the Na^+-dependent uptakes of phenylalanine, aspartic acid, and lysine were influenced very little by 1 mM octylglucopyranoside as compared to the marked inhibition exerted by octylglucopyranoside on Na^+-dependent D-glucose uptake. Moreover Triton X-100, a neutral detegent, strongly inhibited the uptakes of all four metabolites studied. These observations point to a specific interaction between octylglucopyranoside and the Na^+-dependent cotransporter of D-glucose.

Kinetic analysis. We also carried out a kinetic analysis of octylglucopyranoside inhibition at very short uptake times (5 s) [12]. As the uptake is linear up to 10 s, extrapolation to zero-time provides reliable K_i values. Octylglucopyranoside behaves as a competitive inhibitor against D-glucose, when presented in a Hunter and Down-type plot (Fig. 3). The same results were also obtained from Lineweaver and Burk and from Dixon plots.

Parallel kinetic studies with brush-border vesicles from rat and human intestine indicated that octylglucopyranoside always behaves as a competitive inhibitor; the apparent K_i mean values of octylglucopyranoside on D-glucose uptake are 0.035 mM (±0.002 S.E.) for rabbit, 0.1 mM (±0.002 S.E.) for rat and 0.045 mM (±0.007 S.E.) for man. The K_i value for rabbit membrane vesicles is the lowest and is close to that for phlorizin (approx. 7 μM [13]).

To establish whether octylglucopyranoside has a specific action, we measured its uptake in the presence of phlorizin, which has up to now been considered the only effective competitive inhibitor of the Na^+/D-glucose cotransporter and binds at the D-glucose binding sites [5,6]. Applying the Theorell and Semenza plot [14,15] to our data yields parallel lines (Fig. 4), which shows that the two inhibitors compete mutually; these findings suggest the existence of a specific interaction between octylglucopyranoside and the binding site for D-glucose on the Na^+-dependent cotransporter. This result is probably due to the marked structural similarity between D-glucose and octylglucopyranoside. Indeed, octylglucopyranoside

TABLE I

INHIBITION OF Na^+-DEPENDENT AMINO ACIDS AND D-GLUCOSE TRANSPORT INTO BRUSH-BORDER VESICLES BY 1-*O*-*n*-OCTYL-β-D-GLUCOPYRANOSIDE (OGP) AND TRITON X-100 (T)

Membrane vesicles (20 μl) were rapidly mixed at room temperature with 20 μl of the 'uptake buffer' containing 220 μM D-[^{14}C]glucose, or 3 μM L-[^{3}H]phenylalanine, or 20 μM L-[^{3}H]aspartate, or 4 μM L-[^{3}H]lysine and 200 mM NaSCN±2 mM OGP or T. Transport was terminated by addition of 3 ml ice-cold stop solution after 1 min, 8 min or 15 s of incubation as indicated (i.e. at the time of maximum uptake). Mean values and their standard errors for three different experiments performed in triplicate are presented as per cent of the Na^+-dependent uptake inhibition compared to the controls (without OGP or T).

Amino acid / Detergent:	Inhibition (%)	
	OGP	T
L-Phenylalanine (1 min)	25±2 *	99±0.5 **
L-Aspartic acid (8 min)	14±2 *	86±1.5 **
L-Lysine (8 min)	12±1 *	99±3 **
D-Glucose (15 s)	83±4 **	90±2 **

* $P = 0.05$.
** $P < 0.001$.

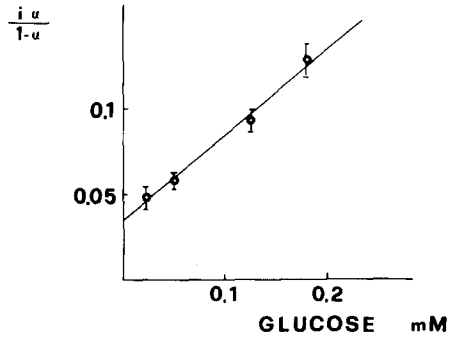

Fig. 3. Hunter and Down plot. Competitive inhibition of D-glucose uptake by octylglucopyranoside into brush-border membrane vesicles. The uptake was carried out with membrane vesicles (20 μl) in 'uptake buffer' containing an initial NaSCN gradient (100 mM out, 0 in) and increasing D-[^{14}C]glucose concentrations (20–200 μM) at constant concentration of 0.2 mM octylglucopyranoside (*i*), $\alpha = v_i/v$. v = pmol D-glucose taken up per mg protein after 5 s of incubation. $v_i = v$ in presence of octylglucopyranoside. The values are the result of one experiment, in triplicate, carried out with the same vesicle preparation.

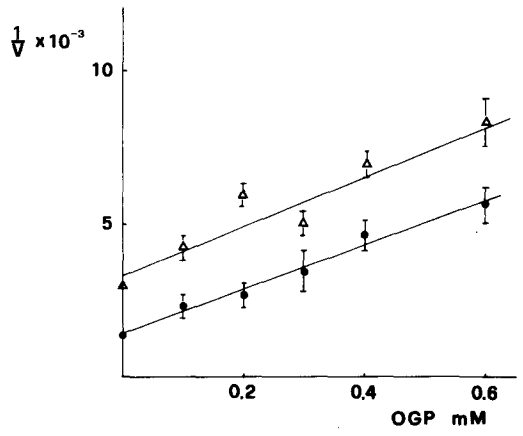

Fig. 4. Dixon plot. Mutual competition between octylglucopyranoside and phlorizin. The experiment was performed with brush-border membrane vesicles (20 μl) in 'uptake buffer' containing 80 μM D-[^{14}C]-glucose, an initial NaSCN gradient (100 mM out, 0 in) and increasing octylglucopyranoside (OGP) concentrations (0.1–0.6 mM) without (●) and with 0.04 mM phlorizin (△). v = pmol D-glucose taken up per mg protein after 5 s of incubation.

is a D-glucose with a hydrophobic octyl chain attached at C-1, while the hydroxyl groups in C-2 and C-6 are both free, as required for binding to the active site of the D-glucose cotransporter [16,17].

Baldwin et al. [18] have reported that octylglucopyranoside does not inhibit the Na^+-independent transport of human erythrocytes even at high concentrations (36 mM); this latter transporter differs from the Na^+-dependent one of brush-border membranes mainly in that the hydroxyl group in position C-1 of glucose is essential for forming the bond between the substrate and the transporter [19].

Moreover, the octyl chain of this amphiphilic detergent may interact with the lipoproteic microenvironment of the D-glucose cotransporter and/or with its hydrophobic surface areas. We suggest that this hydrophobic octylglucopyranoside component is not responsible for the specificity of the inhibition. These interactions, in fact, also occur during comparative studies involving other detergents such as Triton X-100, cholate, deoxycholate, sodium dodecyl sulfate and dodecyltrimethylammoniumbromide [3,20], which, at the membrane level, may show non-specific inhibitory effects on both the Na^+-dependent and independent systems of D-glucose transport and on the Na^+-dependent uptake of amino acids; for an example, see the Triton X-100 inhibition data in Table I.

Acknowledgements

This work was supported by grants from the Italian Consiglio Nazionale delle Ricerche e Ministero della Pubblica Istruzione.

References

1 Crane, R.K., Malathi, P. and Preiser, H. (1977) in Biochemistry of Membrane Transport (Semenza, G. and Carafoli, E., eds.), pp. 261–268, Springer Verlag, Berlin
2 Malathi, P., Preiser, H. and Crane, R.K. (1980) Ann. N. Y. Acad. Sci. 358, 253–266
3 Biber, J., Weber, J. and Semenza, G. (1983) Biochim. Biophys. Acta 728, 429–437
4 Semenza, G., Kessler, M., Hosang, M., Weber, J. and Schmidt, U. (1984) Biochim. Biophys. Acta 779, 343–379
5 Tannenbaum, C., Toggenburger, G., Kessler, M., Rothstein, A. and Semenza, G. (1977) J. Supramol. Struct. 6, 519–533
6 Toggenburger, G., Kessler, M., Rothstein, A., Semenza, G. and Tannenbaum, G. (1978) J. Membrane Biol. 40, 269–290
7 Stevens, B.R., Kaunitz, J.D. and Wright, E.M. (1984) Annu. Rev. Physiol. 46, 417–433
8 Schmitz, J., Preiser, H., Maestracci, P., Ghash, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98–112
9 Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154
10 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
11 Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K.J. (1973) J. Biol. Chem. 248, 25–32
12 Kessler, M., Tannenbaum, V. and Tannenbaum, C. (1978) Biochim. Biophys. Acta 509, 348–359
13 Toggenburger, G., Kessler, M. and Semenza, G. (1982) Biochim. Biophys. Acta 688, 557–571
14 Colombo, V.E. and Semenza, G. (1972) Biochim. Biophys. Acta 288, 145–152
15 Yonetani, T. and Theorell, H. (1964) Arch. Biochem. Biophys. 106, 243–251
16 Barnett, J.E.G., Ralph, A. and Munday, K.A. (1970) Biochem. J. 118, 843–850
17 Barnett, J.E.G., Jarvis, T.S. and Munday, K.A. (1968) Biochem. J. 109, 61–67
18 Baldwin, S.A., Baldwin, J.M. and Lienhard, G.E. (1982) Biochemistry 21, 3836–3842
19 Wheeler, T.J. and Hinkle, P.C. (1985) Annu. Rev. Physiol. 47, 503–517
20 Denk, H. (1979) Pharmac. Ther. 6, 551–577