J. Pharm. Pharmacol. 2000, 52: 303–310
Received March 4, 1999
Accepted August 25, 1999



# Intestinal Transport of β-Thioglycosides by $Na^+$/Glucose Cotransporter

TAKASHI MIZUMA, KATSURA HAGI AND SHOJI AWAZU

*Department of Biopharmaceutics and Drug Rational Research Center, School of Pharmacy, Tokyo University of Pharmacy and Life Science (TUPLS), 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan*

**Abstract**

Intestinal metabolism and transport of *p*-nitrophenyl β-D-thioglucoside (*p*-NPβSglc) and *p*-nitrophenyl β-D-thiogalactoside (*p*-NPβSgal) by the $Na^+$/glucose cotransporter were studied in excised small intestine of the rat.

*p*-NPβSglc and *p*-NPβSgal were stable enough on the mucosal side to be transported to the serosal side. Transport of *p*-NPβSglc was inhibited in the presence of phloridzin (a $Na^+$/glucose cotransporter inhibitor), glucose, or 3-*O*-methylglucose (3-OMG). *p*-NPβSglc transport was dependent on $Na^+$ concentration in a sigmoidal manner. The activation energy for transport was 19·4 kcal $mol^{-1}$. The distribution of transport activity of *p*-NPβSglc in each region of the small intestine correlated well with that of 3-OMG. These results indicate that *p*-NPβSglc is transported by the $Na^+$/glucose cotransporter in small intestine. The order of turnover rate for glycoside transport by $Na^+$/glucose cotransporter was 3-OMG > *p*-nitrophenyl β-*O*-glucoside > *p*-NPβSglc > *p*-NPβSgal, indicating that the presence of a galactose moiety and a sulphur between the monosaccharide moiety and aglycone decreases the turnover rate of the $Na^+$/glucose cotransporter in the transport of glycosides. In an inhibition study using stable *p*-NPβSglc as a $Na^+$/glucose cotransporter-transportable marker glucoside, it was also shown that the $Na^+$/glucose cotransporter recognized several types of glycosides.

In conclusion, displacement of the oxygen at carbon C-1 of glucose by sulphur in thioglycosides decreases the turnover rate of the $Na^+$/glucose cotransporter, but thioglycosides are stable in the small intestine and are transported by the $Na^+$/glucose cotransporter.

The intestinal $Na^+$/glucose cotransporter has been shown to transport glucose and galactose conjugates (Mizuma et al 1992, 1993, 1994). This suggests the applicability of monosaccharide conjugation strategy to intestinal drug delivery by way of the $Na^+$/glucose cotransporter. The order of preference of the $Na^+$/glucose cotransporter for the transport of monosaccharide conjugates is: glucose conjugate > galactose conjugate and β-anomer > α-anomer. However, this information is exclusively limited to *O*-glycosides. To apply the strategy of sugar conjugation to peptides which consist of amino acids that contain sulphur, the intestinal absorption of thioglycosides possessing a thioether bond must be studied. In this paper, therefore, intestinal transport and metabolism of thioglycosides were examined to clarify the requirement of a glycoside bond for $Na^+$/glucose cotransporter-mediated transport, especially the oxygen of the ether bond between C-1 of the glucose moiety and aglycone. Recognition by the $Na^+$/glucose cotransporter of several types of glycoside (Figure 1) was evaluated by studying the inhibitory effect of glycosides on $Na^+$/glucose cotransporter-mediated transport of thioglycosides. The influence of the aglycone moiety on $Na^+$/glucose cotransporter-mediated transport is discussed in relation to intestinal drug delivery.

Correspondence: T. Mizuma, Department of Biopharmaceutics and Drug Rational Research Center, School of Pharmacy, Tokyo University of Pharmacy and Life Science (TUPLS), 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.
E-Mail: mizuma@ps.toyaku.ac.jp

## Materials and Methods

### *Chemicals*

*p*-Nitrophenyl β-D-thioglucoside (*p*-NPβSglc), *p*-nitrophenyl β-D-thiogalactoside (*p*-NPβSgal), *p*-

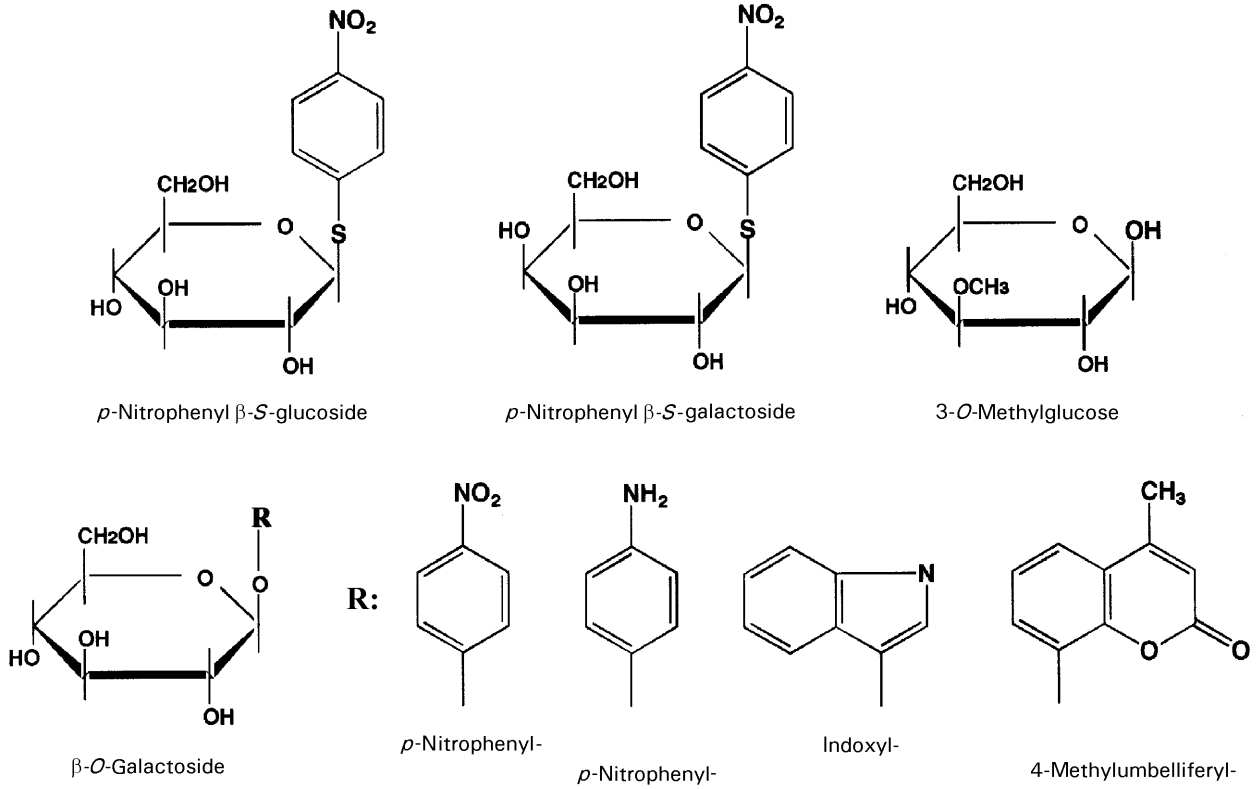


Figure 1. Structure of *p*-nitrophenyl *β*-D-thioglucoside (*p*-NP*β*Sglc), *p*-nitrophenyl *β*-D-thiogalactoside (*p*-NP*β*Sgal) and *β*-*O*-glucosides.

nitrophenyl *β*-D-glucoside (*p*-NP*β*Oglc), *p*-aminophenyl *β*-D-glucoside (*p*-AP*β*Oglc), indoxyl *β*-D-glucoside (IND*β*Oglc), 4-methylumbelliferyl *β*-D-glucoside (4-MU*β*Oglc), D-glucopyranosyl *β*-D-thioglucopyranoside (thiodigluco-side), 3-*O*-methylglucose (3-OMG) and *p*-nitrobenzylalcohol were purchased from Sigma Co. (St Louis, MO). *p*-Nitrobenzylalcohol (*p*-NBA) and glucose assay kit (Glucose test-Wako) were purchased from Wako Pure Chemicals (Tokyo, Japan).

*Intestinal transport*

Intestinal transport was examined in-vitro by previously reported methods (Mizuma et al 1992). Male Wistar rats, 200–250 g (Japan Slc Inc., Shizuoka, Japan), fasted overnight, were anaesthetized with ether and intestinal blood was removed by saline perfusion. The upper, middle or lower part of the small intestine was obtained and everted. The upper part of small intestine (10 cm) was defined as the region that was 2–12 cm below the Treitz ligament. The middle part of the small intestine (10 cm) was defined as the region between points 5 cm above and 5 cm below the half-way point between the Treitz ligament and the ileocaecal junction. The lower part of small intestine (10 cm) was defined as the region that was 2–12 cm above the ileocaecal junction.

Everted small intestine was connected to a disposable 10-mL plastic syringe, and was placed in 30 mL of incubation medium (mM: 113·3 NaCl, 4·83 KCl, 1·214 $KH_2PO_4$, 1·205 $MgSO_4$, 16·96 $NaHCO_3$, 10·18 $Na_2HPO_4$, 0·645 $CaCl_2$, pH 7·4) containing *p*-NP*β*Sglc, *p*-NP*β*Sgal or 3-OMG with *p*-NBA (for correction of practical absorption area) in a beaker through which gas (95% $O_2$, 5% $CO_2$) was bubbled at 37°C in a manner reported previously (Mizuma et al 1992). The serosal side was filled with 5 mL of the incubation medium without *p*-NP*β*Sglc, *p*-NP*β*Sgal or 3-OMG. When necessary, part (or all) of the sodium chloride was displaced with choline chloride. Incubation media (100 *μ*L) were sampled from both the serosal and the mucosal sides every 10 min for 60 min. The samples were mixed with 100 *μ*L of 10% perchloric acid containing 0·5 mM *p*-aminosalicylic acid as an internal standard for high-performance liquid chromatography (HPLC). After centrifugation of the mixture, for 10 min at 1200 rev $min^{-1}$ in a benchtop centrifuge (KM-15200, Kubota, Japan), 25 *μ*L of the resultant supernatant was used for HPLC.

*Metabolic degradation in intestinal homogenates*
Measurement of metabolic degradation of *p*-NP$\beta$Sglc and *p*-NP$\beta$Sgal in intestinal homogenates followed a method reported previously (Mizuma et al 1996, 1997). Ten centimeters of intestine were obtained according to the same methods used for the absorption experiments. The intestine was blotted with filter paper and the wet weight of intestine was measured. The intestine was homogenized with incubation medium using a Physcotron homogenizer (Nichion-Irika, Japan). *p*-NP$\beta$Sglc or *p*-NP$\beta$Sgal was incubated in 2·5% homogenate of intestine at 37°C for 60 min.

*Assay of glycosides and 3-OMG*
Compounds were determined by HPLC. The HPLC system consisted of a pump (880-PU, Jasco Ltd, Tokyo, Japan), UV detector (320 nm, 875-UV, Jasco Ltd), integrator (D-2500, Hitachi, Tokyo, Japan), sample injector (AS-8010, Tosoh, Tokyo, Japan) and an ODS column (TSKgel ODS-80Ts, 6 mm i.d., 150 mm length, Tosoh). The mobile phase consisting of 40% methanol and 0·05% phosphoric acid in water was used at a flow rate of 1·5 mL min$^{-1}$. An assay kit utilizing the *o*-toluidine method was used to determine 3-OMG (Glucose test-Wako).

*Data analysis*
Kinetic parameters for the transport of glycosides were obtained by fitting data to equations 1 and 2 by the nonlinear least-square fitting program, MULTI (Yamaoka et al 1981). The dependency of transport on thioglycosides concentration was analysed by equation 1.

$$v = [(V_{max} \times C)/(Km + C)] + (CLpas \times C) \quad (1)$$

where v represents the transport rate, CLpas represents the transport clearance by passive diffusion, and $V_{max}$ and Km are the maximum rate and the Michaelis constant for Na$^+$/glucose cotransporter-mediated transport, respectively.

Na$^+$-concentration dependency of Na$^+$/glucose cotransporter-mediated transport was analysed by the Hill equation as follows (Kaunitz et al 1982):

$$V_{Na} = (V_{max} \times C_{Na}^n)/(Km_{Na}^n + C_{Na}^n) \quad (2)$$

where $C_{Na}$, $Km_{Na}$ and n are the concentration of Na$^+$, the Michaelis constant and the Hill coefficient, respectively. $v_{Na}$ is Na$^+$-dependent transport rate, which is calculated by subtracting the transport rate in the absence of Na$^+$ from that in the presence of Na$^+$. $V_{max}$ is the maximum rate. The ratio (Rk) of turnover rate of the Na$^+$/glucose cotransporter for glycoside transport to that of 3-OMG was calculated by equation 3.

$$Rk = k, gly/k, 3\text{-}OMG = V_{max}, gly/V_{max}, 3\text{-}OMG \quad (3)$$

where k, gly and k, 3-OMG are the turnover rate for transport of glycosides and 3-OMG, respectively. $V_{max}$, gly and $V_{max}$, 3-OMG are the maximum rate ($V_{max}$) of the transport of glycosides and 3-OMG, respectively. $V_{max}$ (mole unit per time per a unit of length of small intestine) is expressed by equation 4.

$$V_{max} = (n \times k)/\text{Avogadro's number} \quad (4)$$

where n and k represent the number of cotransporters per unit of length of small intestine and the turnover rate of the cotransporter for a round trip between the outside and inside of the cell, respectively. Statistical analysis of the data was performed by Tukey–Kramer or Dunnett's multiple comparisons post test following analysis of variance.

## Results

*Effects of phloridzin and absence of Na$^+$ on serosal appearance of* p *-NP$\beta$Sglc*
Figure 2 shows the concentration–time profiles of *p*-NP$\beta$Sglc on the serosal side. *p*-NP$\beta$Sglc concentration increased with time, but the rate of serosal appearance of *p*-NP$\beta$Sglc decreased in the presence of phloridzin (Na$^+$/glucose cotransporter inhibitor) and in the absence of Na$^+$ (cosubstrate of

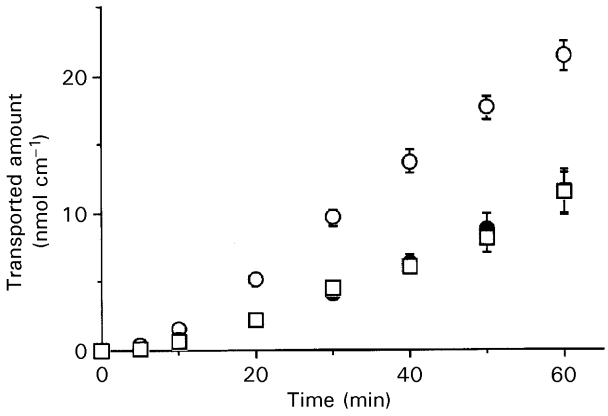


Figure 2. Time-course of serosal appearance of *p*-NP$\beta$Sglc (0·25 mM) following transport across rat small intestine (upper region) under various conditions. Data represent means ± s.e.m. (n = 4–8). Control, ○; phloridzin, ●; Na$^+$-free, □.

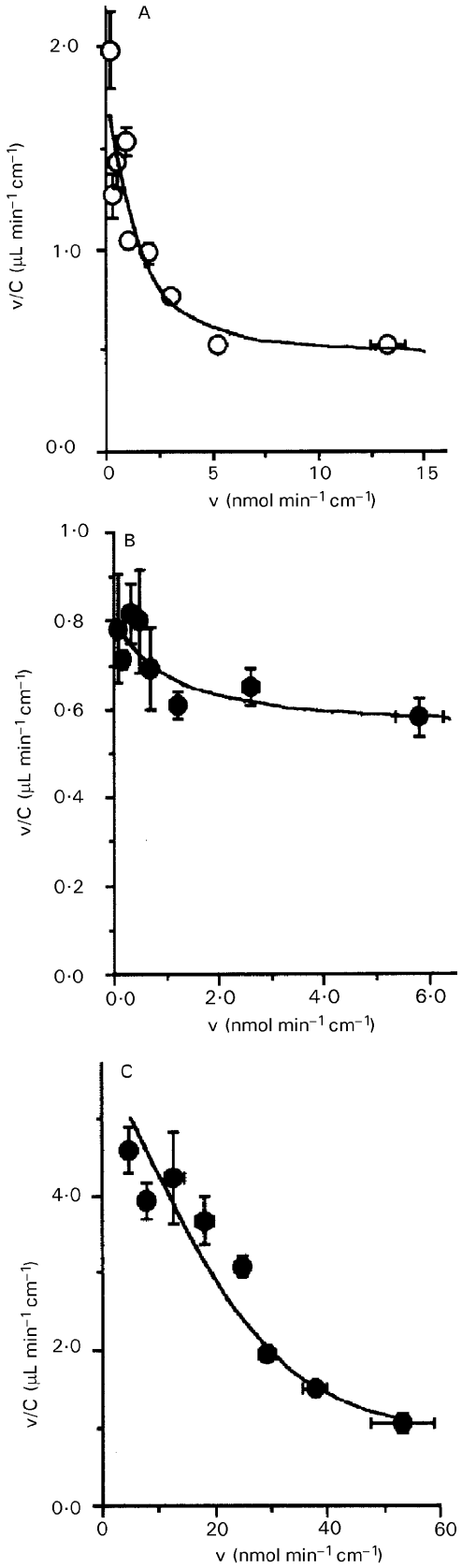


Figure 3. Eadie-Hofstee plots for transport of *p*-NP$\beta$Sglc (A), *p*-NP$\beta$Sgal (B) and 3-OMG (C) across rat small intestine (upper region). Data represent means ± s.e.m., n = 3–8. Curves were obtained by fitting data to equation 1 (see text).

$Na^+$/glucose cotransporter). The concentration of *p*-NP$\beta$Sglc on the mucosal side under each condition did not markedly decrease after 60 min (data not shown). Other compounds were not detected on either the mucosal or the serosal side. Similar results were observed for *p*-NP$\beta$Sgal absorption, although its transport rate was lower than that of *p*-NP$\beta$Sglc (data not shown).

### *Stability of* p-*NPβSglc and* p-*NPβSgal in intestinal homogenates*

The concentration of *p*-NP$\beta$Sglc in the homogenate of the upper part of the small intestine was constant for 60 min, indicating that this thioglucoside was extremely stable even in intestinal homogenates (data not shown). Similar stability was observed for *p*-NP$\beta$Sgal.

### *Concentration dependency of the transport of* p-*NPβSglc,* p *-NPβSgal and 3-OMG*

Figures 3a–c show Eadie–Hofstee plots of the intestinal transport of *p*-NP$\beta$Sglc, *p*-NP$\beta$Sgal and 3-OMG. These figures indicate that the transport of all 3 consists of both saturable and non-saturable components. Kinetic parameters for the transport of 3-OMG and glycosides, which were obtained by equation 1, are summarized in Table 1. The Km values indicated that the order of affinity for the $Na^+$/glucose cotransporter was *p*-NP$\beta$Oglc > *p*-NP$\beta$Sglc > *p*-NP$\beta$Sgal > 3-OMG. The order of $V_{max}$ (turnover rate) was 3-OMG > *p*-NP$\beta$Oglc > *p*-NP$\beta$Sglc > *p*-NP$\beta$Sgal. The order of $V_{max}$/Km values, which represent specific transport activity of $Na^+$/glucose cotransporter-mediated transport, was 3-OMG = *p*-NP$\beta$Oglc > *p*-NP$\beta$Sglc > *p*-NP$\beta$Sgal.

### *Effect of transport inhibitors on* p-*NPβSglc transport*

Table 2 shows the effect of $Na^+$/glucose cotransporter inhibitors on *p*-NP$\beta$Sglc transport. Transport decreased in the presence of $Na^+$/glucose cotransporter-transportable substrate (glucose or 3-OMG) and in the presence of $Na^+$/glucose cotransporter inhibitor (phloridzin). Transport was also inhibited by 2,4-dinitrophenol. These results indicate that *p*-NP$\beta$Sglc is transported by the $Na^+$/glucose cotransporter.

### *Effect of* $Na^+$ *concentration on* p-*NPβSglc transport*

Figure 4 shows the effect of $Na^+$ concentration on *p*-NP$\beta$Sglc transport. The transport was dependent

Table 1. Kinetic parameters of intestinal transport of glucose derivatives in rat intestine.

| | Km (mM) | Vmax (nmol min$^{-1}$cm$^{-1}$) | Vmax/Km ($\mu$L min$^{-1}$ cm$^{-1}$) | Rk | CLpas ($\mu$L min$^{-1}$ cm$^{-1}$) |
|---|---|---|---|---|---|
| 3-OMG | 5·57 ± 1·78 | 29·2 ± 6·45 | 5·240 | 1·0000 | 0·56 ± 0·13 |
| *p*-NP$\beta$Oglc[a] | 0·59 ± 0·18 | 3·02 ± 0·38 | 5·120 | 0·1034 | 0·88 ± 0·04 |
| *p*-NP$\beta$Sglc | 1·11 ± 0·65 | 1·46 ± 0·71 | 1·320 | 0·0500 | 0·45 ± 0·08 |
| *p*-NP$\beta$Sgal | 1·45 ± 0·82 | 0·37 ± 0·49 | 0·255 | 0·0127 | 0·55 ± 0·07 |

Data represent fitted values ± s.d. (except the values of Vmax/Km). [a]Values calculated from the data of Mizuma et al (1992).

on $Na^+$ concentration in a sigmoidal manner, indicating a cooperative effect of $Na^+$ on the $Na^+$/glucose cotransporter-mediated transport of *p*-NP$\beta$Sglc. Nonlinear least-square fitting of data to equation 2 gave n = 1·8 and Km = 28·1 mM.

*Temperature dependency of* p-*NP$\beta$Sglc transport*

Figure 5 shows the Arrhenius plot of $Na^+$/glucose cotransporter-mediated transport of *p*-NP$\beta$Sglc. Transport of *p*-NP$\beta$Sglc by the $Na^+$/glucose cotransporter was dependent on temperature and its activation energy was 19·4 kcal mol$^{-1}$.

*Comparison of Na$^+$/glucose cotransporter-mediated transport of* p-*NP$\beta$Sglc and 3-OMG in the upper, middle and lower regions of small intestine*

Table 3 shows the transport clearance of *p*-NP$\beta$Sglc and 3-OMG in each part of small intestine in the presence and absence of $Na^+$, and $Na^+$/glucose cotransporter-mediated transport clearances. The clearance for $Na^+$/glucose cotransporter-mediated transport of *p*-NP$\beta$Sglc in the upper part of small intestine was nearly the same as in the middle part, but was higher than in the lower part. Similar regional differences were observed for the transport of 3-OMG.

*Inhibition activity of several types of glycosides on the transport of* p-*NP$\beta$Sglc by the Na$^+$/glucose cotransporter*

Table 4 shows the inhibitory effects of *O*-glycosides and *S*-glycosides on the transport of *p*-NP$\beta$Sglc. The transport of *p*-NP$\beta$Sglc was significantly decreased in the presence of *O*-glycosides such as *p*-NP$\beta$Oglc, *p*-AP$\beta$Oglc, IND$\beta$Oglc and 4-MU$\beta$Oglc. The transport of *p*-NP$\beta$Sglc was inhibited by *p*-NP$\beta$Sgal, but not by thiodiglucoside.

## Discussion

The transportability of *p*-NP$\beta$Sglc and *p*-NP$\beta$Sgal across the intestinal barrier to the serosal side was evaluated. *p*-NP$\beta$Sglc and *p*-NP$\beta$Sgal are completely stable in the intestinal tract and intestinal homogenates (data not shown). 3-OMG was also shown to be stable on the mucosal side, although glucose was metabolized on both sides, where the

Table 2. Effects of transport inhibitors on transport of *p*-NP$\beta$Sglc (0·25 mM) across rat small intestine.

| Conditions | Amount transported (nmol/30 min cm$^{-1}$) |
|---|---|
| Control | 9·69 ± 0·39 (8) |
| Glucose (25 mM) | 3·58 ± 0·30* (3) |
| 3-OMG (25 mM) | 5·47 ± 1·31* (4) |
| Phloridzin (1 mM) | 4·19 ± 0·27* (4) |
| 2,4-Dinitrophenol (2 mM) | 7·16 ± 0·36* (4) |

Data represent means ± s.e.m., n = 4–8, indicated in parentheses. *$P < 0·05$, compared with control, obtained by Dunnett's multiple comparison post test following analysis of variance. The upper region of the small intestine was used.

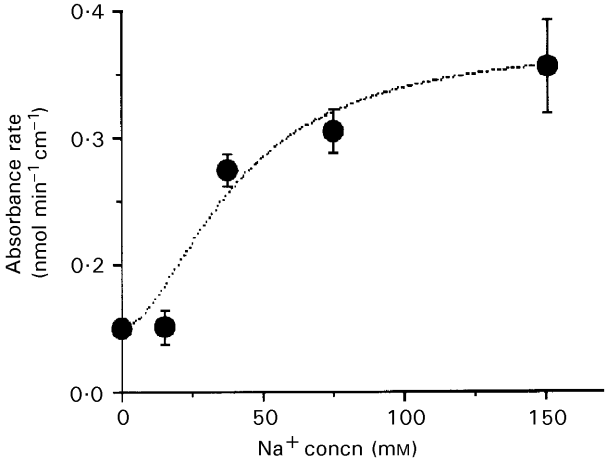


Figure 4. $Na^+$-concentration dependency of *p*-NP$\beta$Sglc (0·25 mM) transport by the $Na^+$/glucose cotransporter of rat small intestine (upper region). The dotted curve was obtained by fitting data to equation 2 (see text). Data represent means ± s.e.m., n = 4–6.

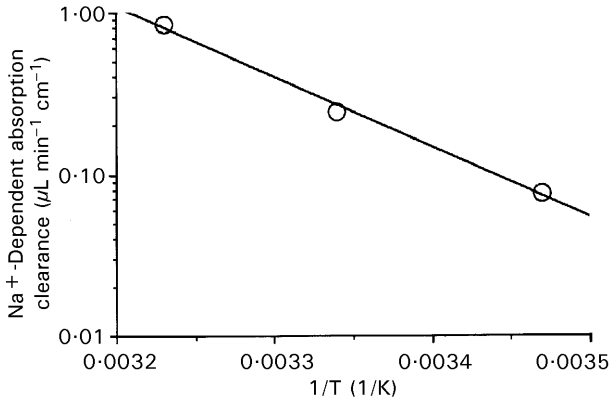


Figure 5. Arrhenius plot of $Na^+$/glucose cotransporter-mediated transport of *p*-NP$\beta$Sglc (0·25 mM) across rat small intestine (upper region). Data represent means (n = 3–8). $Na^+$/glucose cotransporter-mediated transport was calculated by subtracting the amount of *p*-NP$\beta$Sglc transported in the absence of $Na^+$ from the amount transported under control conditions.

elimination clearance of 1 mM glucose on the mucosal side was 42·9 $\mu$L min$^{-1}$ cm$^{-1}$, and the concentrations of glucose on the serosal side after 20 and 60 min were 20 and 12 $\mu$M, respectively (data not shown). Therefore, the transportability of 3-OMG transport was calculated by the same method as the transport of *p*-NP$\beta$Sglc and *p*-NP$\beta$Sgal.

The transport of *p*-NP$\beta$Sglc was concentration dependent (Figure 3a) and decreased in the presence of either $Na^+$/glucose cotransporter inhibitor or $Na^+$/glucose cotransporter-transportable substrates (Figure 2, Table 2). The transport of *p*-NP$\beta$Sglc depended on temperature, and the activation energy for $Na^+$/glucose cotransporter-mediated transport was 19·4 kcal mol$^{-1}$ (Figure 5), approximately comparable with the value reported by Loo et al (1996). These results suggest that *p*-NP$\beta$Sglc is transported by the $Na^+$/glucose cotransporter. The transport rate of *p*-NP$\beta$Sglc versus the concentration of $Na^+$ was sigmoidal (Figure 4), and fitted well to equation 2 based on the Hill equation of $Na^+$ for the $Na^+$/glucose cotransporter (Kaunitz et al 1982). The values for n of 1·8 and Km of 28·1 mM were similar to the reported values for glucose transport (n = 1·9 and Km ~ 38 mM; Kaunitz et al 1982). Similarly, Ikeda et al (1989) reported that n = 1·5 and Km = 32 mM with the oocytes cloned from rabbit small intestine, and n = 1·7 and Km = 52 mM with brush-border membrane vesicles of rabbit small intestine. $Na^+$/glucose cotransporter-mediated transport of *p*-NP$\beta$Sglc was also suggested by an electrophysiological experiment, in which a short-circuit current was induced by *p*-NP$\beta$Sglc, and was inhibited by the presence of phloridzin (Mizuma 1998). Therefore, it is likely that *p*-NP$\beta$Sglc is transported by the $Na^+$/glucose cotransporter.

The Km of *p*-NP$\beta$Sglc was lower than that of 3-OMG or the reported value (2·8 mM) of glucose (Thomson et al 1982), indicating that the affinity of *p*-NP$\beta$Sglc for the $Na^+$/glucose cotransporter was higher than that of 3-OMG. Since the Km of *p*-NP$\beta$Oglc was also lower than that of 3-OMG, we

Table 3. Comparison of absorption clearances in regions of rat small intestine.

| Region | *p*-NP$\beta$Sglc 0·25 mM | 3-OMG 5 mM |
|---|---|---|
| Total | | |
| Upper | 1·43 ± 0·10* | 1·96 ± 0·11* |
| Middle | 1·65 ± 0·17* | 1·92 ± 0·03* |
| Lower | 0·908 ± 0·077 | 0·705 ± 0·058 |
| $Na^+$-dependent | | |
| Upper | 0·855 ± 0·096 | 1·28 ± 0·11* |
| Middle | 1·02 ± 0·17 | 1·23 ± 0·03* |
| Lower | 0·623 ± 0·077 | 0·397 ± 0·058 |
| $Na^+$-independent | | |
| Upper | 0·576 ± 0·020* | 0·682 ± 0·089 |
| Middle | 0·631 ± 0·075* | 0·689 ± 0·202 |
| Lower | 0·285 ± 0·026* | 0·308 ± 0·072 |

Values represent means ± s.e.m., n = 4–8, $\mu$L min$^{-1}$ cm$^{-1}$. *$P < 0·05$, NS for lower region, obtained by Tukey–Kramer multiple comparison post test following analysis of variance.

Table 4. Inhibitory effects of *O*-glycosides and *S*-glycosides (25 mM) on transport of *p*-NP$\beta$Sglc (0·25 mM) across rat small intestine.

| Condition | Absorption clearance of *p*-NP$\beta$Sglc ($\mu$L min$^{-1}$ cm$^{-1}$) |
|---|---|
| Control | 1·43 ± 0·10 (8) |
| *p*-NP$\beta$Oglc | 0·549 ± 0·023* (4) |
| *p*-AP$\beta$Oglc | 0·471 ± 0·052* (3) |
| IND$\beta$Oglc | 0·467 ± 0·058* (3) |
| 4-MU$\beta$Oglc | 0·439 ± 0·010* (3) |
| Thiodiglucoside | 1·48 ± 0·02 (3) |
| *p*-NP$\beta$Sglc | 0·798 ± 0·111* (4) |

*p*-NP$\beta$Oglc, *p*-nitrophenyl $\beta$-D-glucoside; *p*-AP$\beta$Oglc, *p*-aminophenyl $\beta$-D-glucoside; IND$\beta$Oglc, indoxyl $\beta$-D-glucoside; 4-MU$\beta$Oglc, 4-methylumbelliferyl $\beta$-D-glucoside; thiodiglucoside,D-glucosyl $\beta$-D-thioglucoside; *p*-NP$\beta$Sgal, *p*-nitrophenyl $\beta$-D-thiogalactoside. The upper region of the small intestine was used. Data represent means ± s.e.m. (n = 4–8, indicated in parentheses). *$P < 0·05$, vs control, obtained by Dunnett's multiple comparison post tests following analysis of variance.

conclude that aglycone increases the affinity for the $Na^+$/glucose cotransporter. The value of $V_{max}$/Km of *p*-NP$\beta$Oglc was similar to that of 3-OMG, indicating that the specific transport activity of *p*-NP$\beta$Oglc was comparable with that of 3-OMG, and an order of 3-OMG $\cong$ *p*-NP$\beta$Oglc > *p*-NP$\beta$Sglc > *p*-NP$\beta$Sgal.

On the other hand, the Rk (or $V_{max}$) of *p*-NP$\beta$Sglc, *p*-NP$\beta$Sgal and *p*-NP$\beta$Oglc were lower than that of 3-OMG (Table 1), indicating that conjugation of an aglycone to glucose decreases the turnover rate of the $Na^+$/glucose cotransporter. Furthermore, the Rk of glycosides are in the order of *p*-NP$\beta$Oglc > *p*-NP$\beta$Sglc and *p*-NP$\beta$Sglc > *p*-NP$\beta$Sgal, indicating that the presence of a galactose moiety and a sulphur between the monosaccharide moiety and aglycone decreases the turnover rate of the $Na^+$/glucose cotransporter for glycoside transport. These results indicate that any modification of glycosides may affect not only affinity for the $Na^+$/glucose cotransporter but also the turnover rate of the $Na^+$/glucose cotransporter. In a previous study we hypothesized that the $Na^+$/glucose cotransporter might have spare space localized around the $\beta$-position rather than the $\alpha$-position at carbon C-1 of glucose, which is space for aglycone to transport monosaccharide conjugates (Mizuma et al 1998). An NMR study showed that the glucose conformation of *p*-NP$\beta$Sglc was $^4C_1$ chair form, the same as that of *p*-NP$\beta$Oglc (unpublished data). Since the aglycone of *p*-NP$\beta$Sglc and *p*-NP$\beta$Oglc is the same, it is suggested that sulphur changes the conformation of the glycoside to decrease the affinity and the turnover rate. *p*-NP$\beta$Sglc was also transported in the middle and lower regions of the small intestine. The distribution of $Na^+$/glucose cotransporter-mediated transport of *p*-NP$\beta$Sglc was similar to that of 3-OMG, indicating that *p*-NP$\beta$Sglc was absorbed by the $Na^+$/glucose cotransporter in all regions of the small intestine (Table 3). $Na^+$-dependent absorption clearances of *p*-NP$\beta$Sglc were approximately 0·6–1·0 $\mu$L min$^{-1}$ cm$^{-1}$. This value corresponds to half to one-third that of orally active paracetamol (2 $\mu$L min$^{-1}$ cm$^{-1}$, unpublished results).

*p*-NP$\beta$Sglc is UV-detectable, stable in the intestine and transportable by the $Na^+$/glucose cotransporter. However, *O*-glucosides such as *p*-NP$\beta$Oglc and 1-naphthyl $\beta$-*O*-glucoside that were able to be transported by the $Na^+$/glucose cotransporter, were partially desglucosylated (Mizuma et al 1993, 1994). Therefore, *p*-NP$\beta$Sglc was used as a $Na^+$/glucose cotransporter-mediated transportable marker glucoside to study the inhibition of several types of glycoside. The transport of *p*-NP$\beta$Sglc, which was inhibited by $\alpha$-methyl glucose (unpublished result), was inhibited equally by *O*-glucosides and phloridzin, indicating that these glycosides are recognized by the $Na^+$/glucose cotransporter (Table 4). *p*-NP$\beta$Sgal inhibited *p*-NP$\beta$Sglc transport, but thiodiglucoside did not, indicating that aglycone affects the recognition of thioglycosides by the $Na^+$/glucose cotransporter.

In summary, *p*-NP$\beta$Sglc and *p*-NP$\beta$Sgal were shown to be transported by the $Na^+$/glucose cotransporter in small intestine. It was also shown that conjugation of an aglycone to glucose increases the affinity for the $Na^+$/glucose cotransporter, but decreases the turnover rate of the $Na^+$/glucose cotransporter. By using stable *p*-NP$\beta$Sglc as a $Na^+$/glucose cotransporter-transportable marker glucoside, it was shown that the $Na^+$/glucose cotransporter recognized several types of glycoside.

*Acknowledgements*

The authors thank Ms Noriko Hisaki and Mr Yoshiyuki Hosoi for technical assistance.

## References

Kaunitz, J. D., Gunther, R., Wright, E. M. (1982) Involvement of multiple sodium ions in intestinal D-glucose transport. Proc. Natl Acad. Sci. USA 79: 2315–2318

Ikeda, T. S., Hwang, E.-S., Coady, M. J., Hirayama, B. A., Hediger, M. A., Wright, E. M. (1989) Characterization of a $Na^+$/glucose cotransporter cloned from rabbit small intestine. J. Membrane Biol. 110: 87–95

Loo, D. D. F., Zeuthen, T., Chandy, G., Wright, E. M. (1996) Cotransport of water by the $Na^+$/glucose cotransporter. Proc. Natl Acad. Sci. USA 93: 13367–13370

Mizuma, T. (1998) Carrier-mediated oral drug delivery utilizing glucose transporter: importance of turnover rate of transporter in drug delivery. Presented at The Ninth Japanese-American Conference on Pharmacokinetics and Biopharmaceutics, Nagoya.

Mizuma, T., Ohta, K., Hayashi, M., Awazu, S. (1992) Intestinal active absorption of sugar-conjugated compounds by glucose transport system: implication of improvement of poorly absorbable drugs. Biochem. Pharmacol. 43: 2037–2039

Mizuma, T., Ohta, K., Hayashi, M., Awazu, S. (1993) Comparative study of active absorption by the intestine and disposition of anomers of sugar-conjugated compounds. Biochem. Pharmacol. 45: 1520–1523

Mizuma, T., Ohta, K., Awazu, S. (1994) The $\beta$-anomeric and glucose preferences of glucose transport carrier for intestinal active absorption of monosaccharide conjugates. Biochim. Biophys. Acta 1200: 117–122

Mizuma, T., Ohta, K., Koyanagi, A., Awazu, S. (1996) Improvement of intestinal absorption of leucine enkephalin by sugar coupling and peptidase inhibitors. J. Pharm. Sci. 85: 854–857

Mizuma, T., Koyanagi, A., Awazu, S. (1997) Intestinal transport and metabolism of analgesic dipeptides, kyotorphin: rate-limiting factor in intestinal absorption of peptide as drug. Biochim. Biophys. Acta 1335: 111–119

Mizuma, T., Nagamine, Y., Dobashi, A., Awazu, S. (1998) Factors that cause the $\beta$-anomeric preference of $Na^+$/glucose transporter for intestinal transport of monosaccharide conjugates. Biochim. Biophys. Acta 1381: 340–346

Thomson, A. B. R., Hotke, C. A., Weinstein, W. M. (1982) Comparison of kinetic constants of hexose uptake in four animal species and man. Comp. Biochem. Physiol. 72A: 225–236

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn. 4: 879–885