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STUDIES ON THE TRANSPORT OF GLUCOSE FROM DISACCHARIDES BY HAMSTER SMALL INTESTINE IN VITRO
II. CHARACTERISTICS OF THE DISACCHARIDASE-RELATED TRANSPORT SYSTEM***

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SUMMARY

The presence of disaccharidase-associated transport different from the Na^+-dependent monosaccharide transport system in the hamster small intestine is confirmed. The transport of glucose from sucrose is substantially independent of Na^+. In the absence of Na^+, glucose released from sucrose does not mix with a pool ot added free glucose but is directly transferred. Maltose, isomaltose and trehalose acf similarly to sucrose.

Both moieties released from sucrose, glucose and fructose, are transferred. The extent of uptake is not related to total sucrase activity.

The possibility is considered that the brush border disaccharidases may subserve a translocating "carrier" function for a part of the products of their enzymic action.

INTRODUCTION

In a previous paper [1] we reported evidence for the existence of a component of intestinal transport of glucose from a disaccharide substrate which could not be accounted for by the known monosaccharide transport systems [2, 3]. In the present paper, we report further studies of this disaccharidase-related transport with particular emphasis on (1) its occurrence independently of the presence of Na^+ and (2) the transfer of glucose from the disaccharide substrate into the cell without its being mixed with a pool of free glucose added to the bathing medium.

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MATERIALS AND METHODS

The compounds used in this study were obtained from the following commercial sources: [^{14}C]sucrose ([U-^{14}C]glucose) from New England Nuclear Corp.; D-xylose, L-xylose and L-fucose from Pfanstiehl Labs Inc. and all the other compounds from the same sources previously indicated [1]. The analytical and incubation methods used here have been extensively described earlier [1]. However, it is worthwhile briefly reemphasizing that two kinds of intestinal preparations were used for studies of uptake; namely, rings of everted intestine and everted sacs tied to polyethylene tubing. The important difference between the two is that the latter restricts access of the substrates to the brush border pole of the absorbing cells. Incubations were generally carried out with large medium to tissue ratios (50 ml to 0.3 g) at high shaking rates (200 cycles/min) and for short (2 min) time periods in order to minimize the build-up of high concentrations of glucose locally at the tissue surface and in the medium.

For the preparation of Na^+-free Krebs–Ringer phosphate buffer, choline chloride was used for the replacement of NaCl in the modified Krebs–phosphate buffer [4] and potassium phosphates for sodium phosphates.

RESULTS

Independence of Na^+

As is well known [2, 5], Na^+ is required for the intestinal transport of free glucose and our previously reported studies identifying a disaccharidase-related transport system [1] were carried out with Na^+ in the medium. More recently, however, sucrose was tested as a substrate for intestinal preparations in Na^+-free buffers with the kind of result shown in Fig. 1. As seen in this figure, replacement of Na^+ resulted in a reduction in the tissue accumulation of glucose derived from sucrose which is relatively modest in sharp contrast to the nearly total cessation of accumulation from free glucose. It appears that the transport of glucose from sucrose is to a very substantial degree independent of Na^+.

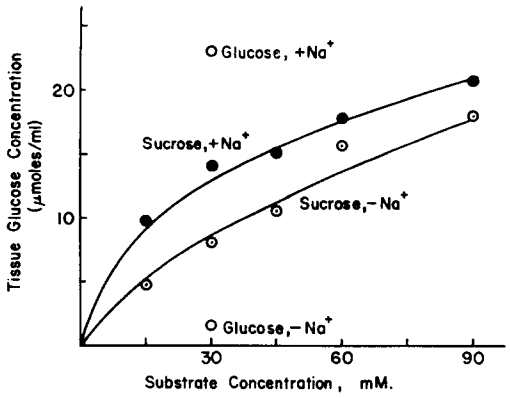

Fig. 1. Uptake of glucose from sucrose in the presence or absence of Na^+. Incubation was in 50 ml of Na^+ or choline$^+$ buffer for 2 min.

The direct nature of glucose transfer

Parsons and Prichard [6] have studied the relationship of hydrolysis of disaccharides to the transport of the resulting glucose with an in vitro perfused preparation from amphibian small intestine. They have concluded that the processes of disaccharide hydrolysis and transfer of the released hexose units are sequential and independent processes and that the hexose product of hydrolysis and free hexose added to the medium contribute to a common pool of hexose and compete for a common step in the overall process. Our studies stand in contrast.

Hamster intestinal preparations were incubated with [^{14}C]glucose-labelled sucrose in the absence of Na^+. Under these conditions (Table I), addition of 30 mM glucose to the medium had no effect on the specific activity of the tissue [^{14}C]glucose

TABLE I

EFFECT OF VARIOUS ADDITIONS ON THE SPECIFIC ACTIVITY OF TISSUE GLUCOSE DERIVED FROM [^{14}C]SUCROSE

Everted segments prepared on polyethylene tubing [1] to prevent substrate access to the serosal surface were incubated for 2 min at 37 °C in 10 ml of choline$^+$ buffer.

Compounds added	Concn (mM)	Tissue glucose (mM)	Specific activity cpm/μmole
[^{14}C]Sucrose	50	6.0	3520
[^{14}C]Sucrose	50+		
+glucose	30	6.3	3660
[^{14}C]Sucrose	50+		
+fructose	30	7.4	2610
[^{14}C]Sucrose	50+		
+maltose	30	9.0	2200
Maltose	30	2.9	—

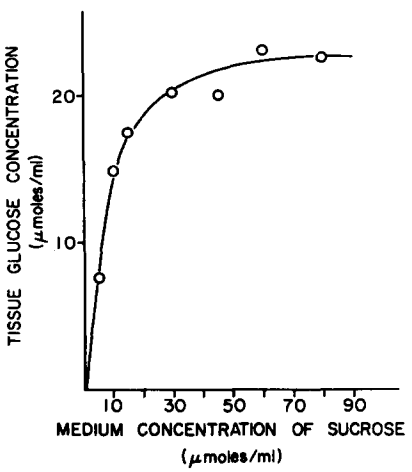

Fig. 2. Uptake of glucose from sucrose in sodium-free medium as a function of concentration. Incubation was in 50 ml of choline$^+$ buffer for 4 min.

derived from the labelled sucrose. Addition of fructose which enters the tissue and is then converted to glucose or of maltose which contributes disaccharidase-related glucose transport similar to but independent of sucrose (see below) reduced the specific activity of the tissue [^{14}C]glucose. This same kind of result with labelled sucrose has been obtained with rings of intestine prepared without the polyethylene tubing. However, the use of sacs was deemed to give clearer results since in these preparations the serosal side was rendered inaccessible to the substrate. With the use of sacs, entry through the serosal side and hydrolysis by an internal disaccharidase, as claimed by Sacktor and Wu [7] could not contribute to the observed values of glucose accumulation.

In the absence of Na^+, tissue accumulation of glucose from sucrose exhibited saturation kinetics (Fig. 2). The initial rate was sustained for no more than 4 min (Fig. 3).

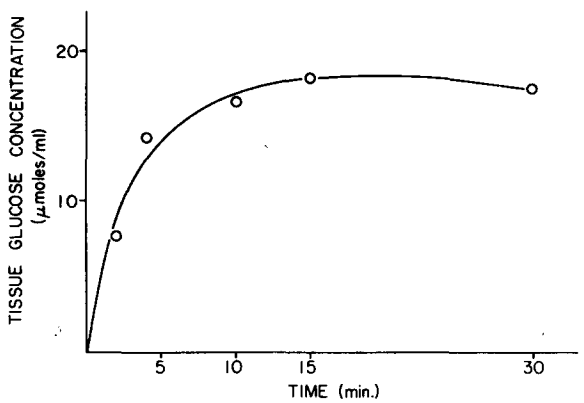

Fig. 3. Uptake of glucose from sucrose in sodium-free medium as a function of time. Incubation was in 50 ml of choline$^+$ buffer with 30 mM sucrose.

TABLE II

UPTAKE OF GLUCOSE FROM DISACCHARIDES IN THE PRESENCE OR ABSENCE OF Na^+

All the substrates were used at 30 mM except trehalose, which was used at 50 mM. Corrections for extracellular glucose were made with phlorizin, where Na^+ buffer was used. In the experiments with disaccharides in choline$^+$ buffer, only corrections for endogenous glucose were made.

Substrate	Tissue glucose (mM)	
	Na^+ buffer	Choline$^+$ buffer
Glucose	20.5	0.83
Sucrose	16.9±1.7	8.4±1.6
Maltose	23.3±3.5	8.5±1.1
Isomaltose	21.4±2.1	8.2±2.9
Trehalose	7.6±1.2	6.9±0.1
Sum from disaccharides	69.5±6.8	31.4±6.0
Sucrose+maltose+isomaltose+trehalose	47.7±4.8	35.2±8.2

Uptake from disaccharide mixtures

Tests with other disaccharides for which hydrolases are known to be associated with the brush border membrane [8] were made to find out (1) whether the intestinal preparations exhibited Na^+-independent components of uptake for all of them and (2) whether there are any interactions between the disaccharides for uptake, in the presence or absence of Na^+.

It can be seen from Table II that with sucrose, maltose and isomaltose a significant amount of glucose uptake occurred in the absence of Na^+ and that with trehalose glucose uptake was almost completely independent of Na^+. The effect of the omission of Na^+ on uptake of glucose from free monosaccharide is included for comparison and uptake is almost completely abolished as expected.

When all the disaccharides were used together as substrates, individual contributions to total tissue glucose were additive in the absence of Na^+ but they were not additive in the presence of Na^+. This difference suggested that the presence of Na^+ introduces some kind of inhibitory interaction. The nature of this inhibitory interaction in the presence of Na^+ is not known but it was demonstrated by use of [^{14}C]glucose-labelled sucrose as in Table III. Maltose inhibited glucose uptake from sucrose by about 80% without uptake from itself being inhibited. Isomaltose inhibited glucose uptake from sucrose and was in turn inhibited to about the same extent. Trehalose was not inhibitory nor was it inhibited.

TABLE III

GLUCOSE UPTAKE FROM DISACCHARIDE MIXTURES IN Na^+ MEDIUM

Period of incubation is 2 min. All the disaccharides were used at 30 mM, except trehalose, which was at 50 mM. Values in brackets represent per cent inhibition of glucose uptake from the individual disaccharides when added in mixture with other disaccharides.

Substrate	Total tissue glucose (mM)	[^{14}C]Glucose from sucrose	Glucose from other disaccharide
Sucrose ([U-^{14}C]glucose)	16.7	17.1	–
Maltose	27.9	–	27.9
Maltose + sucrose ([U-^{14}C]glucose)	31.3	3.4 (79%)	27.8 (0%)
Isomaltose	21.2	–	21.2
Isomaltose + sucrose ([U-^{14}C]glucose)	25.3	10.02 (38%)	15.3 (28%)
Trehalose	10.4	–	10.4
Trehalose + sucrose ([U-^{14}C]glucose)	28.0	17.6 (0%)	10.0 (4%)

Studies with substrates of Na^+-dependent glucose transport and inhibitors of sucrase

It has been reported earlier [1] that β-methylglucoside and D-galactose which are substrates of the Na^+-dependent monosaccharide transport system inhibit non-competitively the uptake of glucose from sucrose. These studies, however, were carried out in the presence of Na^+. In the absence of Na^+ these compounds are inert. Table IV contrasts the two conditions. In the presence of Na^+ glucose uptake but not sucrose hydrolysis was inhibited. In the absence of Na^+, neither hydrolysis nor uptake was affected.

TABLE IV

EFFECT OF MONOSACCHARIDES AND GLUCOSIDES ON SUCROSE HYDROLYSIS AND TRANSPORT OF GLUCOSE FROM SUCROSE

All additions were made at concentrations of 30 mM. Period of incubation is 2 min.

Additions to 30 mM sucrose	Na^+ buffer % inhibition		Choline$^+$ buffer % inhibition	
	Sucrose hydrolysis	Glc uptake	Sucrose hydrolysis	Glc uptake
β-Methylglucoside	0	86	0	0
D-Galactose	0	61	0	0
D-Xylose	82	67	51	47
L-Xylose	43	19	38	14
L-Fucose	48	29	59	24

D-Xylose, L-xylose and L-fucose inhibit sucrase activity in homogenates of intestinal mucosa. Hence, these compounds were tested for their effect on glucose uptake from sucrose in the presence and absence of Na^+ as shown in Table IV. All three inhibited hydrolysis and uptake under both conditions. Inhibition of hydrolysis was to be expected and was found. Inhibition of uptake was also expected as it could be assumed to be related to the effect of the compounds on hydrolysis. However, the differing extents of inhibition of the two processes in two of the three cases would suggest that the situation is more complex. The inhibition of glucose uptake from sucrose is not related to an effect on the monosaccharide transport system in as much as we could expect these compounds, like β-methylglucoside and galactose, to be not inhibitory in the absence of Na^+.

Effect of Tris$^+$

Tris$^+$ is well known to be a potent competitive inhibitor of disaccharidases [9–13]. Hence, studies were done to ascertain the effect of Tris$^+$ on glucose uptake from sucrose or trehalose compared to Na^+ or choline$^+$ and the results are presented in Table V. The presence of Tris$^+$ compared to choline$^+$ gave about 50% further reduction in glucose uptake from sucrose, while it had a much smaller effect on glucose uptake from trehalose. These relative effects are consistent with the relatively

TABLE V

EFFECT OF IONS ON GLUCOSE TRANSFER FROM DISACCHARIDES

The substrates were used at 50 mM. Period of incubation is 2 min. Tris chloride was used to substitute for NaCl in Krebs–phosphate buffer.

Substrates	Tissue glucose (mM)		
	Na^+	Choline$^+$	Tris$^+$
Sucrose	21.5±1.7	13.4±1.8	7.4±0.8
Trehalose	6.7±0.8	5.9±0.5	5.2±0.7

greater effect of $Tris^+$ on sucrase activity in homogenates (see Table VI). However, the reduction in glucose uptake by intact tissue in changing from Na^+ to $Tris^+$ (Table V) is very much smaller than the reduction in hydrolysis by homogenates caused by the same change (Table VI). Clearly, intact tissue retains hydrolytic capacity in the presence of $Tris^+$.

TABLE VI

EFFECT OF $TRIS^+$ ON BRUSH BORDER SUCRASE AND TREHALASE ACTIVITIES IN BUFFERS USED FOR UPTAKE STUDIES

Sucrase and Trehalase were assayed by the method of Dahlqvist [22], using Krebs–Ringer–Na^+ buffer, pH 7.0, and $Tris^+$ buffer, pH 7.0, in the place of sodium maleate buffer, pH 6.5.

	μmoles Glucose formed /min per mg protein	Percent inhibition
Sucrose–Krebs–Ringer–Na^+ buffer	1.36	—
Sucrose–Krebs–Ringer–$Tris^+$ buffer	0.03	98
Trehalose–Krebs–Ringer–Na^+ buffer	0.228	—
Trehalose–Krebs–Ringer–$Tris^+$ buffer	0.05	78

Parsons and Prichard [6] have reported from their studies with amphibian small intestine that in the presence of $Tris^+$ and with maltose as the substrate, the rate of glucose translocation into the vascular effluent was affected to a lesser extent than maltose hydrolysis and suggested that in the presence of $Tris^+$ there was an apparent increase in efficiency with which the intestinal epithelial cells were able to capture the glucose liberated. Also it has been earlier shown by Bosackova and Crane [14] that $Tris^+$ does not penetrate hamster mucosa to more than the extracellular space, as measured by mannitol. Hence, the results here may be taken to indicate that some part of the enzyme activity is shielded and not available to be inhibited by $Tris^+$. If this be so, the shielded enzyme can be assumed to be at the mucosal surface in as much as sacs were used for these experiments.

Relative concentrations of tissue glucose and fructose derived from sucrose

Tissue glucose and fructose were determined after incubating intestinal segments with sucrose in $choline^+$ buffer for 2 and 5 min with the view to seeing whether or not both moieties were transferred. The results are presented in Table VII and show that both glucose and fructose were transferred. If the medium concentrations of glucose and fructose are taken for comparison, both glucose and fructose gave tissue to medium ratios very much higher than unity in confirmation of the early work of Miller and Crane [15]. Glucose accumulation was higher than fructose at both time intervals. However, in the presence of dinitrophenol, the values were more nearly equal. We assume that dinitrophenol serves in this instance to reduce the ATP-dependent conversion of fructose to glucose.

Relation of glucose uptake from sucrose to total sucrase activity

It is known that sucrase activity increases with age of the animals [16]. Hence, studies with hamsters of various ages were conducted to find out whether the extent

TABLE VII

RELATIVE CONCENTRATIONS OF GLUCOSE AND FRUCTOSE IN TISSUE AND MEDIUM AND EFFECT OF 2,4-DINITROPHENOL

2,4-dinitrophenol was used at a concentration of 0.5 mM and sucrose was at 50 mM. Total reducing sugar was assayed by the method of Somogyi [23] and fructose values were calculated as the difference between total reducing sugar and glucose values as measured by the Lloyd and Whelan's modification [24] of the method of Dahlqvist [22].

	Period of incubation (min)	Tissue		Medium	
		Glucose (mM)	Fructose (mM)	Glucose (mM)	Fructose (mM)
Sucrose	2	9.9	3.0	0.64	0.59
Sucrose + 2,4-dinitrophenol	2	6.6	5.1	0.51	0.53
Sucrose	5	20.3	8.2	1.26	0.9
Sucrose + 2,4-dinitrophenol	5	11.9	9.7	1.52	1.24

of transfer of glucose from sucrose was related to total sucrase activity. Hamsters of age in weeks, 2, 3–4, 6 and 9–10, were used for this study and it was found that the total sucrase activity, expressed as glucose released from sucrose increased nearly 10-fold from 15 to 136 μmoles/g wet wt of the tissue. However, glucose uptake from sucrose increased only from 2.9–4.4 μmoles transferred/g wet wt.

Studies with trehalose

Trehalase, in contrast to the other disaccharidases studied, is insensitive to Na^+ [16]. Consequently, studies with trehalose should indicate, by differences in result, whether the interaction of Na^+ with the other hydrolases is of importance in affecting glucose uptake from their respective substrates.

β-Methylglucoside, D-xylose and L-fucose were tested to find out their effects on glucose uptake from trehalose and trehalose hydrolysis, in the presence or absence of Na^+. D-xylose and L-fucose, which inhibit sucrose hydrolysis, did not at all inhibit trehalose hydrolysis, with or without Na^+, as might be expected from the lack of transglucosidase activity for trehalase [16]. Also, in the absence of Na^+, these compounds and β-methylglucoside were without any significant effect on glucose uptake from trehalose. In the presence of Na^+, D-xylose and L-fucose had no significant inhibitory effects. Surprisingly, in the presence of Na^+, β-methylglucoside at 30 mM abolished glucose uptake. In as much as we have seen[1] transfer of glucose from trehalose additive to uptake from free glucose the simple conclusion that the direct transfer of glucose from trehalose is restricted to the Na^+-free conditions would not seem to be allowed. However, an alternative explanation is not in hand.

DISCUSSION

The results presented above confirm the presence of disaccharidase-associated transport. Moreover, it has now been found that glucose released from sucrose is not diluted by added free glucose but is directly transferred. Also Na^+ is not required.

To the extent studied, all brush border disaccharidases exhibit the same phenomenon and, in the absence of Na^+, independently of one another. Curiously, however, in the presence of Na^+, the various transports are not independent. Maltose and isomaltose inhibit glucose transfer from sucrose when Na^+ is present. This phenomenon may be related to the maltolytic activity of sucrase and isomaltase. However, if it is so related, the maltolytic activity should itself be dependent upon Na^+ in a more absolute way than either sucrase or isomaltase. To our knowledge, no test of this proposition has been made.

The results so far obtained and presented in this and the preceding paper [1] limit the choice of possibilities for an explanation of the phenomenon of disaccharidase-related transport. It seems to us that there are two in number. On the one hand, it could be postulated that the brush border possesses specific carriers for disaccharide transport through the mediation of which intact disaccharide might enter the tissue to be split intracellularly. On the other hand, it could be reasoned that the brush border enzymes, in whole or in part, might themselves subserve the translocation function. We do not favor the first possibility. It is generally believed, on good evidence, that the overwhelming proportion of the sucrase, maltase, isomaltase and trehalase activities of the cell are contained in the membrane [8, 17], although the complete absence of these enzymes in the body of the cell has not been proved by the applicable histochemical techniques [18–20]. Sacktor and Wu [7] have concluded that substantial disaccharidase is elsewhere than at the brush border but the activity they find is not more than 1–2% of the brush border capacity for hydrolysis and interpretations other than theirs are in order. On balance, we favor the second possibility, i.e. some hydrolase molecules are organized in the membrane in such a way that they can accept substrate on one side of the membrane and deliver a part of the products on the other. This interpretation would be in keeping with the studies from Semenza's laboratory [21] in which the reconstitution of the sucrase-mediated glucose–fructose transport system in lipid membranes is reported. Purified sucrase–isomaltase complex has been incorporated into black lipid membranes prepared with lipids from various sources. Membranes prepared without sucrase–isomaltase complex were essentially impermeable to sucrose, glucose, fructose and mannitol while those containing the complex had a larger permeability for glucose and fructose derived from sucrose than for free glucose and fructose. The phenomenon was independent of Na^+. These findings, together with the studies presented above, allow of the possibility that our results can be interpreted to indicate a natural phenomenon which is a consequence of the association of disaccharidases with the brush border membrane.

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