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Properties of a Na^+/galactose(glucose) symport system in *Vibrio parahaemolyticus*

Rafiquel I. Sarker [a], Wakano Ogawa [a], Masaaki Tsuda [a], Shuji Tanaka [b], Tomofusa Tsuchiya [a,*]

[a] *Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan*
[b] *Department of Microbiology, Kochi Medical School, Nangoku, Kochi, Japan*

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Abstract

We have investigated galactose transport in a mutant strain of *Vibrio parahaemolyticus* that lacks a glucose-PTS (phospho*enol*pyruvate:carbohydrate phosphotransferase system) and a trehalose-PTS. Cells of the *V. parahaemolyticus* actively transported D-galactose and Na^+ greatly stimulated the transport. Maximum stimulation of D-galactose transport activity was observed at 10 mM NaCl, and Na^+ could be replaced with Li^+. Addition of galactose to the cell suspension under anaerobic conditions elicited Na^+ uptake. Therefore, we conclude that this organism accomplishes galactose transport by a Na^+/solute symport mechanism. Judging from inhibition results, D-galactose, D-glucose and to a lesser extent α-D-fucose are substrates of this transport system. The Na^+/galactose symport system exhibited a high affinity for D-galactose (K_m: 40 μM) and showed a relatively lower affinity for D-glucose (K_m: 420 μM), but the maximum velocities for galactose and glucose transport were almost same (about 52 nmol/min per mg protein). The Na^+/D-galactose symport system was induced by either D-galactose or α-D-fucose, and repressed by D-glucose.

Keywords: Sodium; Galactose; Glucose; Symport; (*V. parahaemolyticus*)

1. Introduction

Sugars are transported into bacterial cells by several mechanisms. Some bacteria employ more than one mechanism for transport of a single sugar substrate [1–3]. In *Escherichia coli*, which is the best characterized microorganism, the phospho*enol*pyruvate:carbohydrate phosphotransferase system (PTS) is a major transport system for many different sugars [4]. In this system, transported sugars are chemically modified to sugar-phosphate. Some sugars are transported by the periplasmic binding protein-dependent transport system (BPD system) at the expense of ATP hydrolysis [5–7]. In the PTS and BPD systems, several proteins are involved in the translocation of substrates through membranes. There are two other sugar transport systems in which a single carrier protein mediates the translocation process. Facilitated diffusion is one of these systems [8]. In this system, a specific substrate moves only along preexisting gradients, and translocation occurs without an energy expenditure. Another system, which is ion-coupled symport system, requires an electrochemical gradient of ions to energize the transport of substrates [9]. H^+ and Na^+ are the major coupling ions in such systems [1,10–14].

Vibrio parahaemolyticus is a gram-negative facultative anaerobic marine bacterium. It can use Na^+ as well as H^+ as coupling cations in energy transduction [15,16]. As in *V. alginolyticus* [17], the respiratory chain of *V. parahaemolyticus* extrudes Na^+ under alkaline conditions [18], thus generating a Na^+ electrochemical potential across the membrane that is the driving force for nutrient transport. An electrochemical potential of Na^+ is established also by the Na^+/H^+ antiporter in this organism [19]. In *Vibrios*, the transport of various nutrients is dependent on Na^+. Most amino acid transport systems and the sucrose transport system in *V. alginolyticus* are Na^+-dependent [12,20], and adenosine transport in *V. parahaemolyticus* is Na^+-dependent [15]. In *V. parahaemolyticus*, however, glucose, fructose, mannose, trehalose and mannitol have been shown to be taken up by the PTS, which is a major transport system for sugars [21]. Kubota et al. isolated a number of

* Corresponding author. Fax: +81 86 2517957; e-mail: tsuchiya@ph2ews1.okayama-u.ac.jp.

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mutants of *V. parahaemolyticus* that were defective in the utilization of different PTS sugars [21,22]. Recently, we found that cells of the mutant strain 1122, which is a glucose-PTS-negative (and trehalose-PTS-negative) mutant, actively transport D-glucose by a Na^+/glucose symport mechanism [23]. Further studies showed that transport of D-glucose was increased by more than 10-fold when cells were grown in the presence of D-galactose. Thus, we were interested in studying the relationship between D-glucose transport system and D-galactose transport system. D-Galactose is an epimer of D-glucose at C-4. In bacterial cells, D-galactose serves not only as an energy source or material for synthesis of cellular components through metabolism, but also as a building block in complex polysaccharide formation. D-Galactose transport systems of *E. coli* have been studied extensively. In *E. coli*, D-galactose is transported mainly by two major galactose transport systems. One of these is a binding protein dependent transport system also called β-methylgalactoside permease (MgIP) [24,25], and the other is galactose permease (GalP), an H^+-linked transport system [1,24–26]. In addition, there are several other non-specific systems. D-Galactose and a non-metabolizable sugar, D-fucose, induce both of these two major galactose transport systems [24,26]. Accordingly, we characterized the D-galactose transport system in *V. parahaemolyticus* strain 1122 and found that D-galactose is also transported by a Na^+/galactose symport mechanism. Here we report properties of the Na^+/galactose (and glucose) transport system of *V. parahaemolyticus*.

2. Materials and methods

V. parahaemolyticus wild type strain 1010 and mutant strain 1122 (lacking both glucose-PTS and trehalose-PTS) [21–23] were used in this study. Cells were grown aerobically at 37°C either in V-5 minimal medium consisting of 50 mM K_2HPO_4, 510 mM NaCl, 10 mM $(NH_4)_2SO_4$, 0.75 mM $MgSO_4$, 0.05 mM $CaCl_2$, 0.025 mM $ZnCl_2$ and 0.025 mM $FeSO_4$ (pH 8.0) [21] supplemented with 20 mM D-galactose, or in YP medium (pH 8.0) [21] consisting of 0.3% yeast extract, 1% polypeptone and 3% NaCl.

For transport assays, cells were grown in the YP medium in the absence or presence of 20 mM D-galactose. In some experiments, 20 mM D-glucose or α-D-fucose was added to the culture medium instead of D-galactose. Cells were harvested in the late exponential phase of growth, washed three times with a buffer consisting of 0.2 M Mops-Tris (pH 7.5), 10 mM $MgSO_4$, 20 mM Tris-lactate, 10 mM NaCl (unless otherwise indicated) and then resuspended in the buffer at a concentration of about 0.1–0.2 mg cell protein/ml. After preincubation at 25°C for 3 min, transport was initiated by the addition of D-[^{14}C]galactose or D-[^{14}C]glucose (0.25 μCi/ml, final concentration 0.1 mM). Samples were taken at intervals, filtered, washed and dried. Radioactivity was counted using a liquid scintillation counter.

For measurement of Na^+ uptake, cells were washed three times with a buffer consisting of 0.2 M Mops-tetramethylammonium hydroxide (TMAH) (pH 7.5) and 10 mM $MgSO_4$, and suspended in the same buffer. Uptake of Na^+ was measured in an assay mixture consisting of 0.2 M Tricine-TMAH (pH 8.5), 0.3 mM NaCl and cells (2–3 mg cell protein/ml), using a Na^+-selective electrode, as described previously [13].

Methyl-α-glucoside, L-glucose, α-D-fucose were from Sigma, St. Louis, MO, USA. D-Glucose, D-galactose, 2-deoxy glucose, methyl-β-galactoside, trehalose, L-arabinose, fructose, mannose, mannitol, sucrose and maltose were from Wako Chemicals, Japan. D-[^{14}C]Galactose and D-[^{14}C]glucose were obtained from Amersham, USA.

Protein contents were determined according to the method of Lowly et al. [27] with bovine serum albumin as standard.

3. Results

Previously, we investigated glucose transport system in *V. parahaemolyticus* using mutant cells (strain 1122) lacking both a glucose-PTS and a trehalose-PTS [23]. We measured growth of the mutant cells on various sugars. During that course of the study, we noticed that 1122 cells grew slower than the parental cells (strain 1010) when D-galactose was added as a sole source of carbon (Fig. 1). This observation suggested that the glucose-PTS (and/or trehalose-PTS) might effect D-galactose transport in *V. parahaemolyticus*. Thus, we used strain 1122 for the study of D-galactose transport in *V. parahaemolyticus*.

First, we compared D-galactose transport activity in 1010 cells (parent) and 1122 cells (mutant). *V. para-*

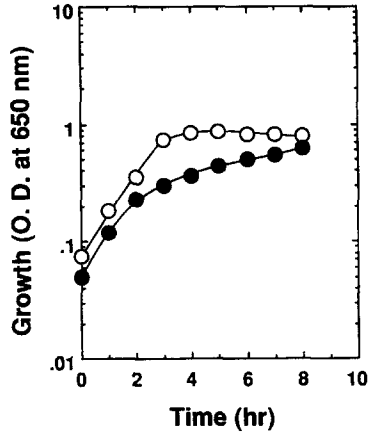

Fig. 1. Growth of *V. parahaemolyticus* wild type and mutant on D-galactose. Wild type cells 1010 (○) and mutant cells 1122 (●) were grown at 37°C with shaking in the minimal medium V-5 supplemented with 20 mM D-galactose. Growth was monitored by measuring turbidity at 650 nm.

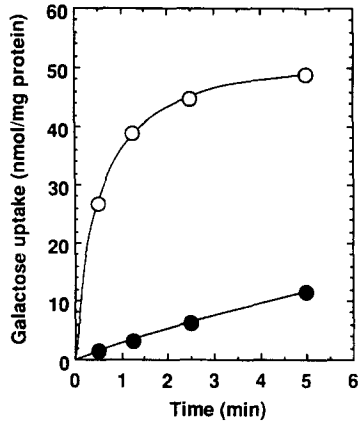

Fig. 2. D-Galactose uptake in wild type cells and in mutant cells of *V. parahaemolyticus*. Cells were grown in YP medium under aerobic conditions (without galactose). Time course of D-[^{14}C]galactose uptake in wild type 1010 (○) or in mutant 1122 (●) is shown.

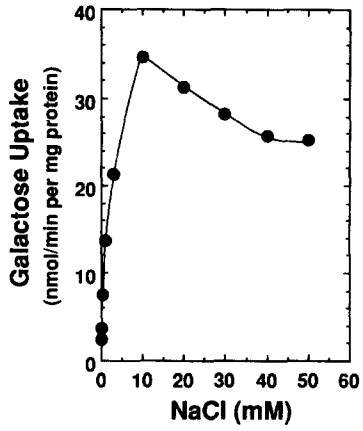

Fig. 3. Effect of Na^+ concentrations on D-galactose transport in *V. parahaemolyticus* mutant 1122. Cells were grown in YP medium supplemented with 20 mM D-galactose under aerobic conditions. Initial velocity of D-[^{14}C]galactose transport was measured at indicated concentrations of NaCl.

haemolyticus 1010 and 1122 cells were grown in the YP medium, and D-galactose transport activity was measured. Since most of the ion-coupled transport systems in *Vibrios* utilize Na^+ as a coupling ion, we measured the D-galactose transport activity in the presence of 10 mM NaCl. Parental cells showed much higher galactose transport activity than the mutant cells (Fig. 2). It has been shown that D-galactose is not a substrate for the PTS in *V. parahaemolyticus* [21]. Thus, it seems that glucose-PTS (and/or trehalose-PTS) effects D-galactose transport indirectly. Previously, we have reported that *V. parahaemolyticus* possesses a Na^+/D-glucose symport system [23]. In that case, transport of D-glucose was greatly stimulated by Na^+. Thus, we tested the effect of Na^+ at various concentrations on D-galactose transport using 1122 cells to avoid the effect of the glucose-PTS (or the trehalose-PTS). Na^+ greatly stimulated D-galactose transport and the maximum transport was observed at 10 mM NaCl (Fig. 3). Above 10 mM NaCl, D-galactose transport activity decreased with increasing NaCl concentrations.

We also tested the effect of various salts on the D-galactose transport (Fig. 4). NaCl and Na_2SO_4 greatly stimulated the galactose transport activity. Stimulation of galactose transport activity was not observed when NaCl was replaced with KCl, Choline-Cl, NH_4Cl, $CaCl_2$, but LiCl stimulated the transport activity. Thus, Na^+ or Li^+, but not Cl^-, stimulates glucose transport. The initial velocity of D-galactose transport was lower with Li^+ than that with Na^+. This result thus suggests that transport of D-galactose couples either to Na^+ or to Li^+, and that the efficiency with Na^+ is higher than that with Li^+.

3.1. Substrate specificity

To determine the substrate specificity of the D-galactose transport system, we tested whether various sugars and sugar analogs inhibit D-galactose transport. An excess (100-fold) of unlabeled test substrate was added 30 s before the addition of D-[^{14}C]galactose to the assay mixture, and the initial velocity of D-galactose transport was measured. When the test substrate was D-galactose, uptake of labeled D-galactose was not observed (data not shown). However, when D-glucose was added, very strong inhibition (96% inhibition) was observed (Table 1). D-Galactose transport was also strongly inhibited (84%) by α-D-fucose, a non-metabolizable sugar. A moderate inhibition (about 58%) was caused by methyl-β-galactoside, an analog of galactose, and by methyl-α-glucoside, an analog of glucose. Addition of other sugars and sugar analogs showed very low or no inhibition in the transport of D-galactose. From the results shown in Table 1, we conclude that the best substrate for this system are (in decreasing order) D-galactose, D-glucose and α-D-fucose.

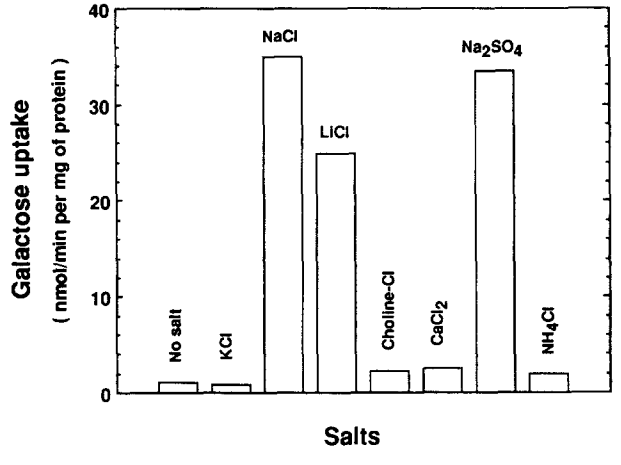

Fig. 4. Effects of various salts on D-galactose uptake. Cells of *V. parahaemolyticus* 1122 were grown in YP medium supplemented with 20 mM D-galactose. Initial velocity of D-[^{14}C]galactose uptake was measured in the absence or presence of 10 mM indicated salt.

3.2. Induction and repression of D-galactose transport system

In the experiments described in Figs. 3 and 4, and Table 1, we used cells (strain 1122) grown in the presence of D-galactose. These cells showed high D-galactose transport activity. However, when 1122 cells were grown in the absence of D-galactose, transport activity was low (Fig. 2). These results suggest that the D-galactose transport system is induced by D-galactose. As shown in Table 1, it seemed that D-glucose and α-D-fucose are also substrates of this transport system. Thus, we investigated the effect of adding these sugars to the growth medium on D-galactose transport activity. As shown in Fig. 5A, D-galactose in the growth medium greatly induced D-galactose transport activity. α-D-Fucose also induced D-galactose transport activity. With regard to the initial velocity of D-galactose transport, cells induced with α-D-fucose showed higher activity than cells induced with D-galactose. The initial velocity of D-galactose transport was about 11 times higher in the galactose induced cells than in uninduced cells. The initial velocity in cells induced with α-D-fucose was about 15 times higher than that in uninduced cells. On the other hand, cells grown in the presence of D-glucose showed lower D-galactose transport activity than cells grown in its absence. Thus, we conclude that D-galactose or α-D-fucose in the growth medium induces the D-galactose transport system and D-glucose strongly suppresses it.

We observed that D-galactose transport was very strongly inhibited by excess D-glucose (Table 1). Previously, we reported that D-glucose transport was strongly inhibited by excess D-galactose [23]. Thus, it seemed likely that D-galactose and D-glucose are transported through the same transport system. To test this possibility, we measured D-glucose transport in cells grown either in the absence or in the presence of D-galactose, α-D-fucose or D-glucose. Cells grown in the absence of these sugars showed low but significant D-glucose transport activity. However, when cells were grown in the presence of either D-galactose or α-D-fucose, D-glucose transport activity was very high (Fig. 5B). As suggested previously [23], D-glucose transport activity was very low when cells were grown in the presence of D-glucose (Fig. 5B). The effects of D-galactose, α-D-fucose and D-glucose on inducing or repressing D-galactose transport activity were very similar to their effects on D-glucose transport activity (Fig. 5). Therefore, it is very likely that D-galactose and D-glucose are transported through the same transport system in *V. parahaemolyticus*.

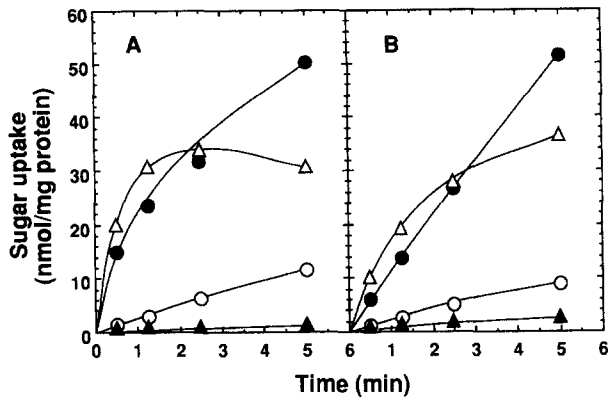

Fig. 5. Induction and repression of galactose transport and glucose transport. Cells of *V. parahaemolyticus* 1122 were grown in YP medium (○), or YP medium supplemented with either 20 mM D-galactose (●), 20 mM α-D-fucose (△) or 20 mM D-glucose (▲). Uptake of D-[^{14}C]galactose (A) or of D-[^{14}C]glucose (B) was measured.

Table 1
Effect of various sugars on accumulation of D-galactose in *V. parahaemolyticus* mutant strain 1122

Sugars added [a]	D-[^{14}C]Galactose uptake [b]	
	nmol/min per mg protein	% of control
No addition	31.8	100
α-D-Fucose	5.1	16
Methyl-β-galactoside	13.3	42
D-Glucose	1.4	4
L-Glucose	30.1	95
Methyl-α-glucoside	13.6	43
Methyl-β-glucoside	30.2	95
2-Deoxyglucose	32.2	101
Fructose	31.9	100
Mannose	32.9	103
Mannitol	32.3	101
Trehalose	27.8	87
L-Arabinose	28.3	89
Maltose	29.5	93
Sucrose	29.5	93

[a] Sugars (unlabeled) were added at 5 mM (100 times excess compared with D-[^{14}C]galactose).
[b] Initial velocity of D-[^{14}C]galactose uptake.

3.3. Kinetics of D-galactose transport

We determined the kinetic parameters of D-galactose transport system in 1122 cells grown in the presence of D-galactose, and investigated the effect of Na^+ on the kinetic parameters. Since the D-galactose transport activity is very low when measured in the absence of Na^+, we measured D-galactose transport at two different concentrations of Na^+, and the K_m values and maximum velocities (V_{max}) were determined. In the presence of 2 mM NaCl, the K_m and V_{max} values for D-galactose transport were 168 μM and 48 nmol/min per mg protein, respectively. At a higher concentration of NaCl, 10 mM, the K_m and V_{max} values were 40 μM and 51 nmol/min per mg protein (Fig. 6). The standard deviations were < 10%. Thus, the V_{max} for D-galactose transport was almost the same at different concentrations of Na^+, but the K_m varied with the Na^+ concentration. These kinetic parameters indicate that the affinity of the transporter for D-galactose is greatly influenced by the Na^+ concentration.

Previously we reported kinetic parameters for D-glucose

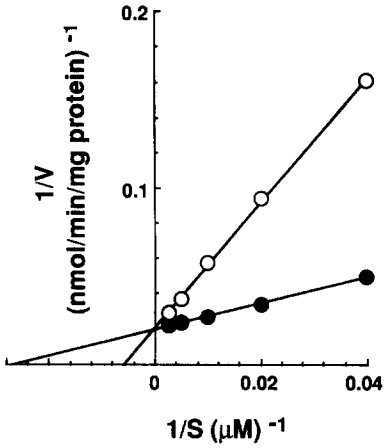

Fig. 6. Lineweaver-Burk plot of Na^+-dependent D-galactose transport in *V. parahaemolyticus* 1122. Cells were grown in YP medium supplemented with 20 mM D-galactose under aerobic conditions. Initial velocity of D-[^{14}C]galactose transport was measured at various concentrations of D-galactose with either 2 mM NaCl (○) or 10 mM NaCl (●).

transport in cells of *V. parahaemolyticus* 1122 grown in the absence of D-galactose [23]. Since D-glucose transport activity is greatly induced with D-galactose, we measured the kinetic parameters of D-glucose transport in cells grown in the presence of galactose, and investigated the effect of Na^+ on the kinetic parameters. In the presence of 2 mM NaCl in the assay mixture, the K_m and V_{max} values for glucose transport were 1.1 mM and 53 nmol/min per mg protein, respectively (Fig. 7). At 10 mM NaCl, K_m and V_{max} were 0.42 mM and 52 nmol/min per mg protein, respectively. The standard deviations were < 10%. Thus, V_{max} for D-glucose transport was almost same at different concentrations of Na^+, but K_m varied with Na^+ concentrations, similar to the case of D-galactose transport. Comparison of the kinetic parameters of cells that were induced with D-galactose [23] with those of uninduced cells indicates that the K_m values are in the same range in the two

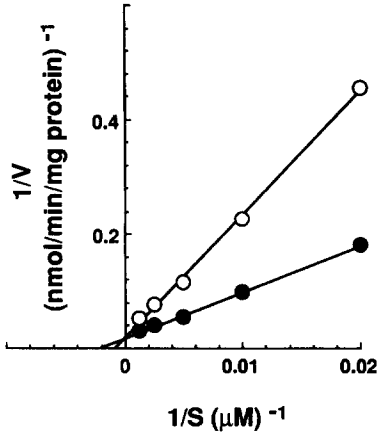

Fig. 7. Lineweaver-Burk plot of Na^+-dependent D-glucose transport in *V. parahaemolyticus* 1122. Cells were grown in YP medium supplemented with 20 mM galactose under aerobic conditions. Initial velocity of D-[^{14}C]glucose transport was measured at various concentrations of D-glucose with either 2 mM NaCl (○) or 10 mM NaCl (●).

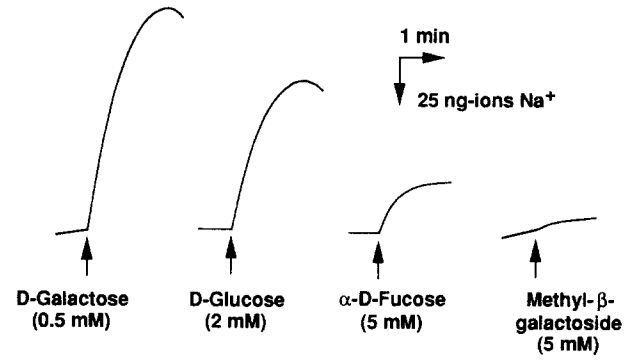

Fig. 8. Na^+ uptake elicited by sugar influx in *V. parahaemolyticus* 1122. Cells were grown under aerobic conditions in YP medium supplemented with 20 mM D-galactose. Arrows indicate times at which an anaerobic solution of each sugar was added to an anaerobic cell suspension to a final concentration shown in the parentheses. Na^+ uptake was measured with an Na^+-selective electrode. Upward deflection represents uptake of Na^+.

types of cells, but that the V_{max} values are roughly 4-fold larger in cells grown in the presence of D-galactose. This indicates that the number of transport proteins increased in cells induced with D-galactose.

3.4. Na^+ uptake elicited by substrate influx

As shown above, D-galactose, D-glucose and α-D-fucose seem to be common substrates for the galactose transport system of *V. parahaemolyticus*, and transport of D-galactose and D-glucose is Na^+-dependent. We previously reported that D-glucose transport in the mutant 1122 of *V. parahaemolyticus* occurred by a Na^+/glucose symport mechanism [23]. If transport of D-galactose and α-D-fucose occurs by the Na^+-coupled symport mechanism, then a Na^+ uptake elicited by galactose (or fucose) influx should be detected. In fact, we detected a Na^+ uptake in galactose-induced cells of 1122 when D-galactose, D-glucose or α-D-fucose was added to the cell suspension under anaerobic conditions (Fig. 8). Thus, we conclude that D-galactose and α-D-fucose, in addition to D-glucose, are actually transported by a Na^+/substrate symport mechanism. The order of the initial velocity of Na^+ uptake elicited by sugar addition was D-galactose > D-glucose > α-D-fucose. Methyl-β-galactoside also caused a slight uptake. These results support the idea that, among the substrates tested, the best substrates were D-galactose, D-glucose and α-D-fucose, in that order.

We then tested whether these substrates were transported via the same transport system. Addition of 2 mM galactose resulted in a velocity of 43 ng-ions Na^+ uptake/min per mg cell protein. When Na^+ uptake (upward deflection) reached a plateau, glucose was added to the same cell suspension to a final concentration of 5 mM, but further uptake of Na^+ was not observed (Fig. 9). A similar result was obtained when α-D-fucose was added as a second substrate after the addition of D-galactose. We investigated the effects of Na^+ uptake when the substrates

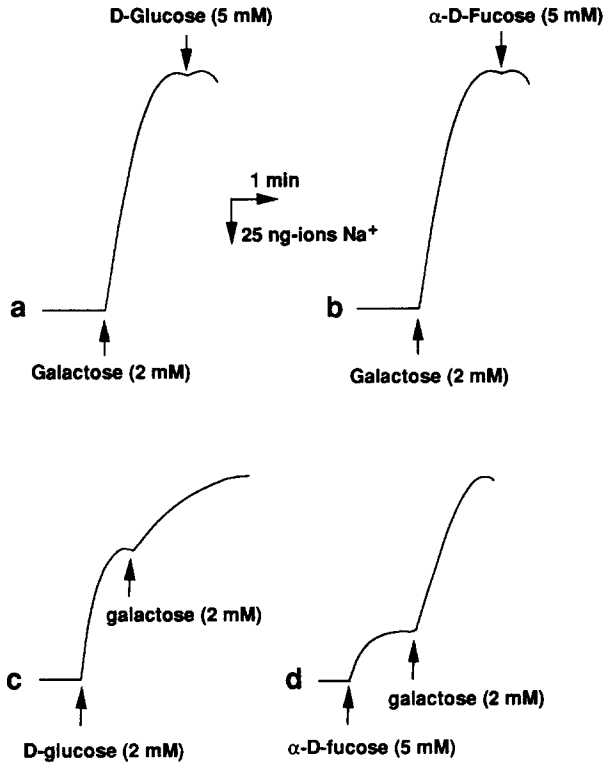

Fig. 9. Competition of Na^+ uptake elicited by sugar influx in *V. parahaemolyticus* 1122. Cells were grown under aerobic conditions in YP medium supplemented with 20 mM D-galactose. Arrows indicate times at which an anaerobic solution of each sugar was added to an anaerobic cell suspension to a final concentration shown in parentheses. Uptake of Na^+ was measured with an Na^+-selective electrode. Upward deflection represents uptake of Na^+.

were added in reverse order. Addition of 2 mM glucose to the cell suspension resulted in a velocity of 21 ng-ions Na^+ uptake/min per mg protein. When upward deflection caused by Na^+ uptake reached a plateau, addition of 2 mM galactose as the second substrate resulted in a further uptake of Na^+ with a velocity of 7.5 ng-ions/min per mg protein (Fig. 9). Similarly, addition of 5 mM α-D-fucose to the anaerobic cell suspension resulted in a velocity of 9.6 ng-ions Na^+ uptake/min per mg protein, and when Na^+ uptake reached a plateau, addition of 2 mM galactose to the same cell suspension resulted in a velocity of 29 ng-ions Na^+ uptake/min per mg protein (Fig. 9). These results indicate several points: (1) galactose, glucose and fucose are transported through the same transport system, (2) transport of these substrates is coupled to Na^+ and (3) the affinities for the substrates are in the order D-galactose > D-glucose > α-D-fucose.

4. Discussion

Na^+-coupled symport systems use the energy of an electrochemical potential of Na^+ to actively transport a substrate. Although these symporters are abundant in animal cells, they are now known to be common in bacteria that grow under a wide variety of conditions [28,29]. In marine bacteria, Na^+ is the main coupling ion for the transport of nutrients. In *V. parahaemolyticus*, many sugars are transported by the PTS, but D-galactose is a non-PTS sugar [21]. *V. parahaemolyticus* can grow on galactose as a sole carbon source, which means that it possesses a high D-galactose uptake activity. In order to conduct a detailed investigation of D-galactose transport, we used *V. parahaemolyticus* mutant strain 1122 whose glucose-PTS and trehalose-PTS are defective. Cells of the mutant strain 1122 actively transported galactose when Na^+ was present in the assay mixture. We also observed Na^+ uptake elicited by the influx of D-galactose. Thus, our results revealed that *V. parahaemolyticus* cells possess a Na^+/D-galactose symport system. It has been shown previously in *E. coli* cells that Li^+ could replace with Na^+ in the melibiose transport system [30] and in the proline porter I [31], both of which are Na^+/substrate symporters. Similarly we have shown that Li^+ could replace with Na^+ to some extent in the Na^+/glucose symport system of *V. parahaemolyticus* [23]. We have also shown that Li^+ is a substrate for the Na^+/H^+ antiporter of *V. parahaemolyticus* [19]. In the present study, we found that Li^+ stimulated transport of D-galactose in *V. parahaemolyticus*, which suggests that Na^+ could be replaced with Li^+ in the Na^+/galactose symport. In *V. alginolyticus*, however, it has been reported that Li^+ could not substitute for Na^+ in Na^+/aminoisobutyric acid symport [20].

Previously, we analyzed the Na^+/glucose symporter in *V. parahaemolyticus* [23]. However, we now believe that Na^+/galactose symport and Na^+/glucose symport are mediated by the same transport system in *V. parahaemolyticus*. The reasons are as follows. (1) Both Na^+/galactose and Na^+/glucose symport activities were induced by D-galactose (or α-D-fucose) and repressed by D-glucose. (2) Transport of D-galactose was strongly inhibited by D-glucose, and that of D-glucose was strongly inhibited by D-galactose. (3) Na^+ influx elicited by D-glucose was inhibited by D-galactose, and vice versa. Judging from the results of kinetic analyses (Figs. 6 and 7) and the results of the competition experiment (Table 1 and Fig. 9), D-galactose is the best substrate followed by D-glucose, and perhaps by α-D-fucose. The sugar analogs methyl-α-glucoside and methyl-β-D-galactoside inhibited D-galactose transport only moderately, and thus seem to be poor substrates. In *E. coli*, galactose, glucose, D-fucose and 2-deoxyglucose are substrates of GalP [24,26], and D-glucose and 2-deoxyglucose are better substrate than D-galactose for this system [26]. In this organism, methyl-β-D-galactoside is a substrate for MglP, but not for the GalP, for which it is complete inhibitor [32]. We are not sure whether methyl-α-glucoside and methyl-β-D-galactoside are true substrates for the Na^+/D-galactose symporter of *V. parahaemolyticus*. 2-Deoxyglucose is not a substrate for this system.

In *V. parahaemolyticus*, the Na^+/D-galactose transport system is inducible. Our result indicates that D-galactose and α-D-fucose induced the transport system. In *E. coli*, both of the two major galactose transport systems (GalP and MglP) are induced by D-galactose and D-fucose [24,26]. Furthermore, D-fucose is a gratuitous inducer of all *gal* operons, and thus D-fucose is useful for maintaining high levels of expression of GalP and MglP in cultures of *E. coli* [24,33]. In *V. parahaemolyticus*, although α-D-fucose is a substrate of the Na^+/D-galactose symporter, this non-metabolizable sugar is a gratuitous inducer of this transport system. It should be noted that D-galactose in the growth medium did not have a significant effect on D-galactose transport activity in the wild type strain (data not shown). Thus, it seems that the glucose-PTS (and/or trehalose-PTS) affects the inducibility of the D-galactose transport systems in *V. parahaemolyticus*. At present, it is not clear how many galactose transport systems are present in wild type cells of *V. parahaemolyticus*. Judging from the observation that wild type cells grown in the absence of galactose showed very high galactose transport activity, other galactose transport system (constitutive system) seems to be present in the wild type cells, and the system would not be present (or activity is very low) in the mutant 1122. Many carbohydrate transport systems in bacteria are subject to control by catabolite repression [34,35] or inducer exclusion [36]. Many metabolic enzymes and transport proteins are repressed when cells are grown in the presence of glucose. The inducible Na^+/D-galactose symport system of *V. parahaemolyticus* is strongly repressed when grown in the presence of D-glucose.

We are unaware of any other reports in the literature on Na^+-dependent galactose (glucose) transport systems in bacteria. Mammalian Na^+/glucose transport systems (SGLT1) have been reported to transport glucose and galactose [37,38]. The H^+/galactose symporter (GalP) of *E. coli* has been sequenced and deduced amino acid sequence showed a significant similarity to several proton-linked sugar transporters (AraE and XylE) [3,32,39]. Interestingly, these transporters also have a significant similarity with the mammalian passive glucose transporter [32]. Therefore, we are very much interested in comparing the Na^+/galactose(glucose) transport system of *V. parahaemolyticus* with the mammalian Na^+/glucose(galactose) transporter (SGLT1) at the molecular level. Recently we have succeeded in cloning the gene encoding the Na^+/galacose(glucose) transporter of *V. Parahaemolyticus*. Sequencing of the gene is now under way.

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