Uphill Transport of Saccharides across an Anion-Exchange Membrane

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Unmodified carbohydrates were transported across an anion-exchange membrane from a neutral solution cell (Part O) to a basic solution cell (Part A) against their concentration gradients. The amount of each sugar transported across the membrane tended to decrease along with an increase in the pK_a value of the sugar. The transportation was dependent on the presence of a hydroxide-ion concentration gradient. These results suggest the uphill transport of sugars based on a coupled counter-transport of hydroxide ion through the anion-exchange membrane. A comparison of the transportation of monosaccharides with that of disaccharides showed that the steric bulkiness of the sugar molecules affected the amount of each sugar transported. Among the water-soluble organic solvents which have a membrane swelling effect, acetonitrile, having the larger dielectric constant, was found to be a highly effective cosolvent to facilitate uphill transport. When aqueous KOH (10 mM) containing D-fructose (2 mM) was added to Part A and a solution of D-fructose (2 mM) in a 3 : 7 mixture of water and acetonitrile was added to Part O, the dialysis at 20 °C for 3 h changed the ratio of the amount of D-fructose in Part A to the amount of D-fructose in Part O from 50 : 50 to 72 : 28.

The active and selective transport of various electrolytes through artificial membranes has attracted much attention as models of biological transport. Although many methods and membranes have been developed for the transportation of various ionic compounds against their concentration gradients,[1] there have been few studies on the uphill transport of the so-called nonelectrolytes.[2] Nonelectrolytes in such studies were modified into ionized derivatives and then transported. The results of a study on the separation and purification of carbohydrates,[3,4] in which the electrodialysis of sugar–borate complexes was compared with dialysis, prompted the present study concerning the transport of sugars without prior transformation into ionized derivatives. Regarding the present study, we report on the uphill transport of unmodified carbohydrates via coupled counter-transport of hydroxide ion across an anion-exchange membrane and an increased permeation of carbohydrates upon the addition of acetonitrile as a cosolvent.

Experimental

Materials. The following solutions and suspensions for the enzymatic analysis of sugar were purchased from Boehringer Mannheim GmbH: triethanolamine (TEA) buffer, ATP, β-NADP, hexokinase (HK)/glucose-6-phosphate dehydrogenase (G6P-DH), glucose phosphate isomerase (PGI), α-glucosidase, G6P-DH/PGI, phosphomannose isomerase, citrate/β-galactosidase buffer, β-galactose dehydrogenase, assay cocktail solution, and invertase. All of the sugars and solvents used in the present study were of reagent grade, and were purchased from Nacalai Tesque. D-Fructose, D-glucose, and D-mannose were enzymatically determined to be contaminated with approximately 2% of their respective isomers.

Apparatus and a Representative Procedure. Uphill transport of carbohydrates was carried out in a dialysis apparatus (Fig. 1) consisting of two compartments, as follows. An alkaline-solution cell (Part A) was separated from a neutral-solution cell (Part O) by an anion-exchange membrane (AR-43 (an experimental membrane supplied by Asahi Glass Co., Ltd.): composition = poly(styrene-*co*-divinylbenzene), thickness = 0.08 mm, ion-exchange capacity = 1.67 mequiv g^{-1} dry memb.)[4] The effective area of the membrane was 12.5 cm^2. Aqueous solutions of D-fructose (2 mM, 95 ml) and KOH (10 mM, 95 ml) containing D-fructose (2 mM) were added to Part O and Part A, respectively (1 M = 1 mol dm^{-3}). After dialysis at 20 °C for 3 h, the amounts of D-fructose in Part O and Part A were determined by enzymatic analysis.[5] The following mixture was prepared: TEA buffer (1.00 ml), ATP solution (0.10 ml), β-NADP solution (0.10 ml), sample solution (0.02 ml), water (1.90 ml), and HK/G6P-DH suspension (0.02 ml). After 15—20 min, the absorbance of the mixture at 339 nm became constant (absorbance A_1), at which time a PGI suspension (0.02 ml) was added and

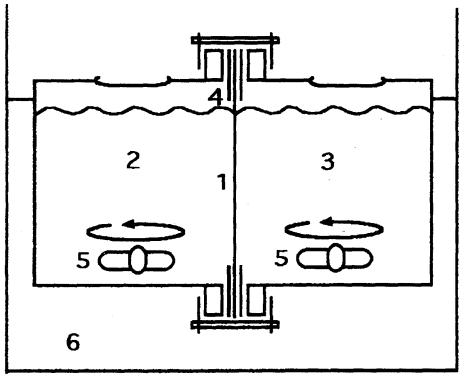

Fig. 1. Apparatus. 1: anion-exchange membrane (AR-43); 2: alkaline solution cell (Part A, 95 ml); 3: neutral solution cell (Part O, 95 ml); 4: rubber packing; 5: stirring bar; 6: water bath.

the absorbance at 339 nm was measured to yield absorbance A_2. The amount of D-fructose was calculated from $A_2 - A_1 = \Delta A$, as previously described.[5] The determination of the amount of the isomerized compounds, D-glucose and D-mannose, was performed analogously.

In a similar manner, the uphill transport of a variety of saccharides in a mixture of water and organic cosolvents was carried out and analyzed using this enzymatic method.[5] Notable: exceptions were the *O*-trimethylsilyl derivatives of D-ribose and glycerol, which were determined using gas chromatography, as reported previously.[6] The determination of isomerized sugars was carried out only in the cases of D-fructose, D-glucose, and D-mannose. Organic solvents in the sample solution did not affect the enzymatic analysis.

Solvent Content in the Anion-Exchange Membrane. The anion-exchange membrane (AR-43) was soaked in a solvent, such as water or a 3 : 7 mixture of water and acetonitrile. After 3 h, the wet weight of the membrane (W_1) was measured. The dry weight of the membrane (W_2) was obtained by weighing it after drying in vacuo at room temperature for 24 h. The solvent content was calculated from the following Eq. 1:

$$\text{Solvent content (\%)} = (W_1 - W_2)/W_2 \times 100. \quad (1)$$

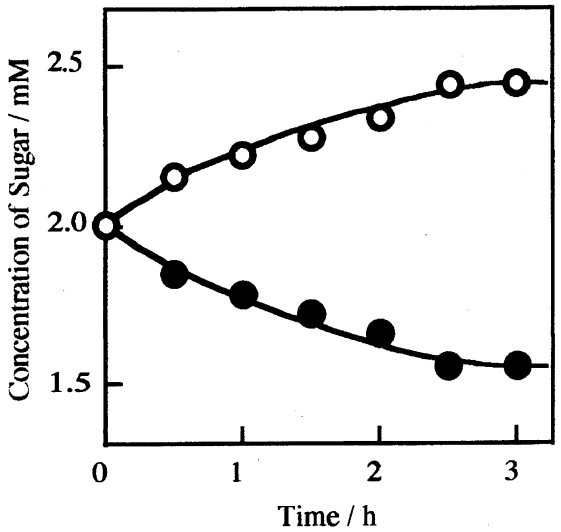

Fig. 2. Time-course for uphill transport of D-fructose. Initial conditions of Part A: [KOH] = 20 mM, [D-fructose] = 2 mM; Part O: [D-fructose] = 2 mM; 3 h; 20 °C. ○, amount of sugar in Part A; ●, amount of sugar in Part O.

Results and Discussion

Uphill Transport of Various Sugars. In a preliminary study we found that D-fructose permeated through an anion-exchange membrane in the presence of a concentration gradient of KOH.[4] Based on this finding, we examined the transportation of various saccharides against their concentration gradients, as shown in Table 1. When 10 mM aqueous KOH containing sugar (2 mM) was placed in Part A and an aqueous sugar solution (2 mM) in Part O, small amounts of the sugars were transported from Part O to Part A during a 3-hour dialysis. An indication of the amount of sugar transported may be seen in the change in the ratio of the D-fructose concentration in Part A to that in Part O from 50 : 50 to 55 : 45. When the KOH concentration in Part A was 20 mM, greater amounts of various saccharides moved across the anion-exchange membrane. For instance, the time-course of uphill transport of D-fructose (shown in Fig. 2) reacted nearly the equilibrium position after 3 h. Among the monosaccharides (Entries 1, 3, 4, 6, and 7 in Table 1), D-fructose and D-mannose, which have smaller pK_a values, were transported efficiently. The amounts of moved sugar transported across the membrane tended to decrease with an increase in the pK_a values[7] of the carbohydrates.

As a kinetic feature, a comparison of Entry 2 with Entry 3 exemplifies the effect of the steric bulkiness of carbohydrates upon uphill transport. After 3-hour dialysis, the amount of D-mannose transported from Part O to Part A was larger than that of maltose, while the pK_a value of D-mannose was slightly greater than that of maltose. A comparison of Entry 5 with Entry 6 shows little difference between the transport facility of lactose and that of D-glucose when the initial concentration of saccharides was dilute (2 mM). However, D-glucose was transported more efficiently than the larger sugar, lactose, when the dialysis was carried out using a 20 mM sugar solution (Part O) and 100 mM aqueous KOH containing 20 mM sugar (Part A). On the other hand, permeation of glycerol, a small size alditol having a large pK_a

Table 1. Uphill Transport of Various Sugars in Water[a]

Entry	Sugar	pK_a[b]	[Sugar in Part A] : [Sugar in Part O]		
			[KOH in Part A] = 10 mM	20 mM	100 mM
1	D-Fructose	12.03	55 : 45	62 : 38	74 : 26[c]
2	Maltose	12.05	54 : 46	57 : 43	62 : 38[c]
3	D-Mannose	12.08	57 : 43	61 : 39	73 : 27[c]
4	D-Ribose	12.11	56 : 44	61 : 39	71 : 29[c]
5	Lactose	12.22	53 : 47	57 : 43	60 : 40[c]
6	D-Glucose	12.28	54 : 46	58 : 42	67 : 33[c]
7	D-Galactose	12.35	52 : 48	55 : 45	67 : 33[c]
8	Sucrose	12.67	51 : 49	53 : 47	58 : 42[c]
9	Glycerol	14.15			50 : 50[c]

a) Initial conditions of Part A: [KOH] = 10, 20, or 100 mM, [sugar] = 2 mM; Part O: [sugar] = 2 mM. Dialysis time, 3 h; temperature, 20 °C; recovery of sugar ≥ 96% (isomers ≤ 4%). b) Ref. 7. c) Initial concentration of sugar in Part A and O, 20 mM. Recovery of sugar, 86—99% (isomers ≤ 5%).

value, through the anion-exchange membrane was not observed (Table 1, Entry 9). Therefore, both the pK_a value and the size of the sugar affected the uphill transport.

Mechanistic Aspects of the Uphill Transport. As a control experiment, D-glucose was subjected to transport under various dialysis conditions, as shown in Table 2. Without the addition of KOH (Entries 1,2, and 3), no transportation of D-glucose took place, and a concentration gradient of neutral salt, KCl, did not affect the transport. A comparison of Entry 4 and Entry 5 indicates that the concentration of hydroxide ion in Part A should be higher than that in Part O. These results and the effect of sugar's pK_a values mentioned above suggest that the uphill transport of carbohydrates via a coupled counter-transport of hydroxide ion across the anion-exchange membrane occurred (Fig. 3). Although saccharides should be in the dissociated form in order to permeate the anion-exchange membrane, saccharides hardly dissociate in Part O. Therefore, saccharides might be deprotonated at the boundary layer between the anion-exchange membrane and Part O, and then permeate the membrane. In support of this conclusion, the amount of sugar transported from Part O to Part A increased, when the KOH concentration in Part A increased from 10 to 100 mM (Table 1).

Effect of Various Organic Solvents. In the transport mechanism proposed in Fig. 3, deprotonation of the hydroxy groups of saccharides in the boundary layer of the anion-exchange membrane seems to be a determining factor. However, the utilization of a highly concentrated KOH solution may lead to an increase in isomerization and decomposition of carbohydrates. In support of this hypothesis, the recovery of D-fructose after a 3-hour dialysis, which was greater than 96% when the KOH concentration in Part A was 20 mM (Table 1, Entry 1), decreased to 89% when 100 mM KOH was used. For accelerating the uphill transport under mild conditions, organic solvents having a membrane swelling effect would be expected to facilitate the movement of sugar through the polymer chain network of the membrane. When the anion-exchange membrane was soaked in water for 3 h, the solvent content was 15%. In contrast, organic solvents such as acetonitrile, acetone, isopropyl alcohol, and methanol showed a greater solvent content (20—25%). Therefore, water-soluble organic solvents were investigated as cosolvents, while the alkaline concentration in Part A remained dilute. For example, the addition of acetonitrile (Table 3, Entries 1—6) was found to facilitate the uphill transport of D-fructose, compared with an experiment carried out only in water (Table 1, Entry 1, KOH = 10 mM). The amount of D-fructose transported increased as the amount of added acetonitrile increased. A comparison of Entries 2, 4, and 6 with Entries 1, 3, and 5 in Table 3 indicates that the addition of acetonitrile only to Part O is as effective as the addition of acetonitrile to both Parts O and A. Moreover, the recovery of D-fructose in Entries 1, 3, and 5 was lower than that in Entries 2, 4, and 6 in Table 3. The addition of acetonitrile to an alkaline solution cell (Part A) might promote undesired chemical transformations of D-fructose, therefore, organic solvents were added only to the neutral solution cell (Part O) in the experiments outlined below.[8] When the ratio of water to acetonitrile was smaller than 1 : 9, some of the saccharides were not completely soluble. Consequently, the effect of various cosolvents was studied using a water-to-cosolvent ratio of 3 : 7.

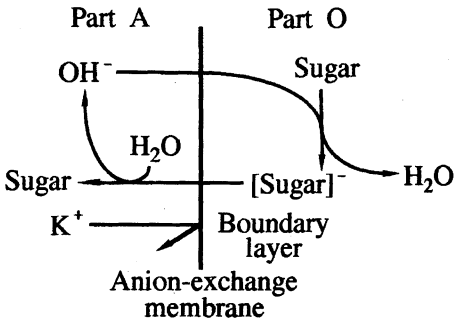

Fig. 3. Proposed mechanism for uphill transport.

Figure 4 shows the effect of cosolvents on the amount of D-fructose transported from Part O to Part A.[9] Among the water-soluble organic cosolvents, such as acetonitrile, acetone, isopropyl alcohol, and methanol, which showed a larger solvent content compared with water, and which would have an advantage to enlarge the membrane channels through which sugars were transported, acetonitrile facilitated the uphill transport most effectively, possibly due to the higher dielectric constant,[10] which is advantageous in the deprotonation process of saccharides in Fig. 3. Although the dielectric constant of water is highest in Fig. 4, the amount of D-fructose transported in water was smaller than that in aqueous acetonitrile, because water was disadvantageous in the membrane swelling effect. This method was used to stimulate the trans-

Table 2. Effect of KOH Concentration Gradient on the Transport of D-Glucose in Water[a]

Entry	Initial concentration (mM)						Concentration of D-glucose[b]
	Part A			Part O			Part A : Part O
	D-Glucose	KOH	KCl	D-Glucose	KOH	KCl	
1	0	0	0	20	0	0	0 : 100
2	0	0	0	20	0	100	0 : 100
3	0	0	100	20	0	0	0 : 100
4	0	100	0	20	0	0	53 : 47
5	0	0	0	20	100	0	8 : 92

a) Dialysis time, 3 h; temperature, 20 °C. b) Recovery of D-glucose, 86—100% (isomers≤5%).

Table 3. Uphill Transport of Sugars in Mixed Solvent[a)]

Entry	Sugar	H_2O/MeCN (v/v)		Concentration of sugar
		Part A	Part O	Part A : Part O
1[b)]	D-Fructose	7/3	7/3	58 : 42
2	D-Fructose	10/0	7/3	56 : 44
3[b)]	D-Fructose	5/5	5/5	64 : 36
4	D-Fructose	10/0	5/5	64 : 36
5[b)]	D-Fructose	3/7	3/7	69 : 31
6	D-Fructose	10/0	3/7	72 : 28
7	Maltose	10/0	3/7	72 : 28
8	D-Mannose	10/0	3/7	69 : 31
9	D-Ribose	10/0	3/7	67 : 33
10	Lactose	10/0	3/7	72 : 28
11	D-Glucose	10/0	3/7	71 : 29
12	D-Galactose	10/0	3/7	69 : 31
13	Sucrose	10/0	3/7	61 : 39

a) Initial conditions of Part A: [KOH] = 10 mM, [sugar] = 2 mM; Part O: [sugar] = 2 mM. Dialysis time, 3 h; temperature, 20 °C; recovery of sugar≥96% (isomers≤3%). b) Recovery of sugar, 91—92% (isomers, 4—6%).

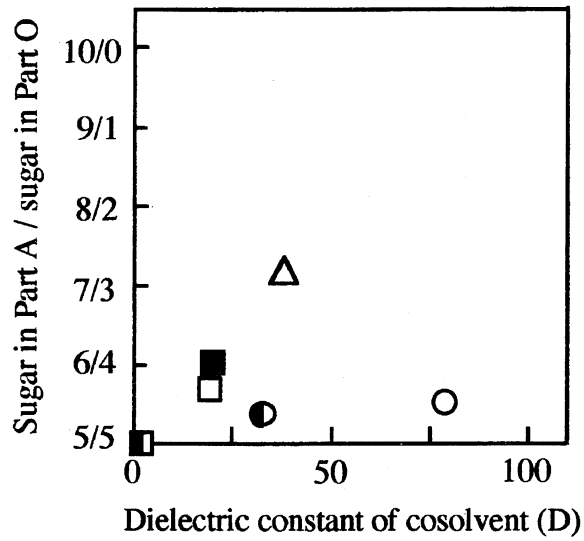

Fig. 4. Uphill transport of D-fructose in a mixed solvent. Part A: [KOH] = 10 mM, [D-Fru] = 2 mM;Part O: [D-Fru] = 2 mM; 3 h; 20 °C. Solvent: Part A, H_2O; Part O, H_2O : cosolvent = 3 : 7. Cosolvent: △, CH_3CN; ■, acetone; ○, H_2O; □, *i*-PrOH; ◐, MeOH; ◧, 1,4-dioxane.

portation of various saccharides. As demonstrated in Entries 6 to 13 in Table 3, the utilization of a 3 : 7 mixture of water and acetonitrile as the Part O solvent and 10 mM aqueous KOH as the Part A solvent facilitated the uphill transport of sugar to a much greater degree compared with dialysis using only water as the Part O solvent and 20 mM aqueous KOH as the Part A solvent, as shown in Table 1. In addition, the recovery of sugars using this method was greater than 96% due to the low concentration (10 mM) of the aqueous KOH used.

Interestingly, the effect of the sugar size on carbohydrate transportation shown in Table 1 was not observed when acetonitrile was used as a cosolvent (Table 3, Entries 7 and 8). Because the solvent content in the anion-exchange membrane increased from 15 to 22% along with an increase in the acetonitrile content in the solvent from 0 to 70%, a possible explanation for the increased permeation of disaccharides upon the addition of acetonitrile is that the aqueous acetonitrile caused an enhanced swelling of the anion-exchange membrane, which resulted in enlarged channels through which disaccharides were transported as well as monosaccharides.

We have demonstrated a novel approach to transport and concentrate unmodified sugars using an anion-exchange membrane. In the method developed in the present study, the concentration gradient of hydroxide ion was essential for, and the addition of an organic solvent having a membrane swelling effect as well as a large dipole moment promoted, the uphill transport of various saccharides.

We are indebted to Asahi Glass Co., Ltd. for the generous gift of the experimental membrane, AR-43.

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8) Although a small amount of acetonitrile permeated through the anion-exchange membrane, HPLC analysis after the dialysis (Table 3, Entry 6) indicated that the content of acetonitrile in Part A was only 4 vol%.

9) Although the final volume of Part O was 12—13 ml greater than that of Part A when the cosolvent was dioxane, the difference in volume after a 3-h dialysis was only 4—5 ml when other cosolvents were used.

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