CHEMISTRY LETTERS, pp. 433-436, 1990.

Uphill Transport of Carbohydrates across Ion-Exchange Membranes

Yoshihiro SHIGEMASA,* Shin-ichi OKAMOTO,
Hitoshi SASHIWA, and Hiroyuki SAIMOTO
Department of Materials Science, Faculty of Engineering,
Tottori University, Tottori 680

Without any transformation into ionized derivatives, carbohydrates themselves were transported across ion-exchange membranes against their concentration gradients. Amounts of moved sugars were affected by their pKa values.

In biological transport, various organic and inorganic compounds are actively transported across biomembranes. In the past few decades, many methods have been developed for the transportation of metal and organic ions against their concentration gradients.[1)] There have been, however, few studies on uphill transport of so-called nonelectrolytes through artificial membranes.[2)] Nonelectrolytes in such studies were converted into ionized derivatives and then were transported. In this communication, we want to report uphill transport of carbohydrates mediated by ion-exchange membranes without any transformation into ionized sugar complexes.

At first we compared uphill transport with electrodialysis of carbohydrates. The electrodialysis was carried out by the following Method A.[3)] The anodic cell (Part A) and the cathodic cell (Part C) were separated from the sample cell (Part O) by an anion-exchange membrane (AR-43)[4)] and a cation-exchange membrane (Selemion CMV).[4)] An aqueous solution of sugar (0.02 mol dm^{-3}, 95 ml) containing boric acid (0.04 mol dm^{-3}) and KOH (0.02 mol dm^{-3}), aqueous KOH (0.1 mol dm^{-3}, 95 ml), and aqueous HCl (0.1 mol dm^{-3}, 95 ml) were added to Part O, A, and C, respectively. After the electrodialysis under the applied voltage of d.c. 8 V at ca. 20 °C for 3 h, amounts of moved and remaining sugars were determined by the phenol-sulfuric acid method[5)] or enzymatic analysis.[6)] Uphill transport of carbohydrates was examined by Method B with the same apparatus but without the use of electrodes under the same conditions as in Method A. Method C is same as Method B except that only a sugar solution (0.02 mol dm^{-3}) was added to Part O.

As shown in Table 1, we found that sugars could be transported across the anion-exchange membrane by dialysis (Method B) as well as by electrodialysis[7)] (Method A). It might be assumed that the difference between the movement of D-fructose and that of D-glucose was due to the character of the sugar-borate

Table 1. Transport of Sugar-Borate Complexes[a)]

Sugar	Method	Remaining sugar(%)	Moved sugar(%)
D-Fructose	A	8	85
	B	20	80[b)]
D-Glucose	A	36	59
	B	41	57[c)]

a) Method A: Part A, aq KOH (0.1 mol dm^{-3}, 95 ml); Part O: an aq solution (95 ml) of sugar (0.02 mol dm^{-3}), boric acid (0.04 mol dm^{-3}), and KOH (0.02 mol dm^{-3}); Part C, aq HCl (0.1 mol dm^{-3}, 95 ml); d.c. 8 V; free current; ca. 20 °C; 3 h. Method B: same conditions as Method A but without the use of electric energy; ca. 20 °C; 3 h. Amounts of sugars were determined by the phenol-sulfuric acid method. b) Enzymatic determination: D-Fru(78%)+D-Glc(2%). c) Enzymatic determination: D-Glc(53%)+D-Fru(4%).

complexes.[8)] Of special interest is the fact that ca. 80% of D-fructose and ca. 60% of D-glucose moved from Part O to Part A as a result of uphill transport even without the use of electric energy.

Interest in establishing a novel concept for the (uphill) transport of nonelectrolytes through ion-exchange membranes motivated us to carry out the dialysis without any additives to form ionized sugar derivatives in Part O. As shown in Table 2, we found that the various saccharides moved from Part O to Part A. The amounts of the moved sugars decreased with an increase in their pKa values.[9)] Indeed less than 10% of glycerol was transported in spite of its small

Table 2. Transport of Various Sugars[a)]

Run	Sugar	pKa[b)]	Remaining sugar(%)	Moved sugar(%)
1	D-Fructose[c)]	12.03	37	56[d)]
2	D-Mannose	12.08	44	56
3	D-Xylose	12.15	39	53
4	D-Glucose[c)]	12.28	51	47[e)]
5	D-Galactose	12.35	57	42
6	Glycerol[c)]	14.15	93	7
7	Maltose	12.05	71	17
8	Sucrose	12.62	79	13
9	Raffinose[c)]	12.74	79	19

a) Method C: Part A, aq KOH (0.1 mol dm^{-3}, 95 ml); Part O, an aq solution of sugar (0.02 mol dm^{-3}, 95 ml); Part C, aq HCl (0.1 mol dm^{-3}, 95 ml); ca. 20 °C; 3 h. Amounts of sugars were determined by the phenol-sulfuric acid method. b) Ref. 9. c) Enzymatic determination. d) D-Fru(52%)+D-Glc(4%). e) D-Glc(43%)+D-Fru(4%).

Table 3. Transport of D-Fructose under Various Conditions[a]

Run	Part A KOH(mol dm^{-3})	Part O KOH(mol dm^{-3})	Remaining sugar(%)	Moved sugar(%)
1	0	0	95[b]	2[b]
2	0.1	0.1	45[c]	42[d]
3	0	0.1	87[e]	12
4	0.1(KCl)	0	95	2
5	0.1	0	37	56[f]
6[g]	0.1	0	26	69[h]

a) Method C: see Table 2. Amounts of sugars were determined by Enzymatic analysis. b) Determined by the phenol-sulfuric acid method. c) D-Fru(38%)+D-Glc(7%). d) D-Fru(34%)+D-Glc(8%). e) D-Fru(80%)+D-Glc(7%). f) D-Fru(52%)+D-Glc(4%). g) 30 °C. h) D-Fru (54%)+D-Glc(15%).

molecular size (run 6). Although pKa values of sugars are much higher than pK values of electrolytes such as acids, bases, and salts, the results mean that easiness in deprotonation from the hydroxyl groups of sugars is one of the main factors in controlling the passage of sugars through the anion-exchange membrane. On the other hand, large size disaccharides and a trisaccharide mainly remained at Part O (runs 7-9). Uphill transport was observed in runs 1-3. A concentration gradient of hydroxide ion might be a driving force in the movement of sugars.

In order to give insight into this transportation mechanism, dialysis under various conditions were studied as shown in Table 3. Comparison of runs 1-3 and run 5 shows that concentration of hydroxide ion in Part A must be higher than that in Part O for uphill transport of sugars. A concentration gradient of a neutral salt (run 4) did not cause the transport of D-fructose. These results suggest that sugars are transported by the coupled counter-transport of hydroxide ion through the anion-exchange membrane. Moreover uphill transport of D-fructose was accelerated at the higher temperature (run 6), but the dialysis should be performed at 20 °C in order to prevent isomerization of sugars.[10]

Further studies are in progress on selective uphill transport of carbohydrates. We are grateful to the Ministry of Education, Science, and Culture of Japan (Grant-in-Aid for Scientific Research No. 61303011) and the Asahi Glass Foundation for Industrial Technology for financial support.

References

1) For example: J. H. Moore, and R. S. Schecter, Nature, 222, 476 (1969); C. F. Reusch and E. L. Cussler, AIChE J., 19, 736 (1973); E. M. Choy, D. F.

Evans, and E. L. Cussler, J. Am. Chem. Soc., 96, 7085 (1974); M. Yoshikawa, Y. Imashiro, Y. Yatsuzuka, K. Sanui, and N. Ogata, J. Membr. Sci., 23, 347 (1985); M. Higa, A. Tanioka, K. Miyasaka, ibid., 37, 251 (1988); E. L. Cussler, R. Aris, and A. Bhown, ibid., 43, 149 (1989).

2) G. Brown, D. Thomas, and E. Selegny, J. Membr. Biol., 8, 313 (1972); E. Selegny and J. C. Vincent, Biophys. Chem., 12, 107 (1980); J. C. Vincent and E. Selegny, ibid., 26, 45 (1987); T. Shinbo, K. Nishimura, T. Yamaguchi, and M. Sugiura, J. Chem. Soc., Chem. Commun., 1986, 349; T. Shinbo, Maku (Membrane), 13, 153 (1988); M. Igawa and M. R. Hoffmann, Chem. Lett., 1988, 597.

3) The apparatus used has been described previously and solutions in the three compartments were stirred during electrodialysis: Y. Shigemasa, K. Tanaka, N. Wakasaki, R. Nakashima, H. Sashiwa, and H. Saimoto, Chem. Express, 3, 695 (1988); Y. Shigemasa, S. Okamoto, Y. Sasagawa, R. Nakashima, H. Sashiwa, and H. Saimoto, ibid., 3, 771 (1988).

4) We are indebted to Asahi Glass Co., Ltd. for a generous gift of Selemion CMV and an experimental material, AR-43.

5) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, Anal. Chem., 28, 350 (1956).

6) "Methods of Enzymatic Analysis," 3rd ed, ed by H. U. Bergmeyer, Verlagg Chemie, Weinheim (1984).

7) A study was reported on preferential separation of fructose from glucose by electrodialysis using ion-exchange membranes: K. Venkatasubramanian, S. M. Jain, and A. J. Giuffrida, U.S. Patent 4 299 677 (1981).

8) A. B. Foster, Adv. Carbohydr. Chem., 12, 81 (1957); G. L. Roy, A. L. Laferriere, and J. O. Edwards, J. Inorg. Nucl. Chem., 1957, 106; E. F. Walborg, Jr., D. B. Ray, and L. E. Ohrberg, Anal. Biochem., 29, 433 (1969); S. Aronoff, T. C. Chen, and M. Cheveldayoff, Carbohydr. Res., 40, 299 (1975); R. J. Ferrier, Adv. Carbohydr. Chem. Biochem., 35, 31 (1978).

9) J. J. Christensen, J. H. Rytting, and R. M. Izatt, J. Chem. Soc., B, 1970, 1646; "The Merck Index," 10th ed, ed by M. Windholz, Merck & Co., Inc., Rahway, N. J. (1983); "Lange's Handbook of Chemistry," 13th ed, ed by A. J. Dean, McGraw-Hill Book Company, New York (1985).

10) Enzymatic determination showed that isomerization of D-fructose in Part A produced less than 4% of D-glucose and 0.1% of D-mannose during the transport at 20 °C.

(Received December 13, 1989)