Tetrahedron Letters, Vol. 34, No 49, pp. 7841-7844, 1993
Printed in Great Britain

0040-4039/93 $6.00 + 00
Pergamon Press Ltd

Metal Cation:Glucopyranoside Co-Transport Through a Liquid Organic Membrane

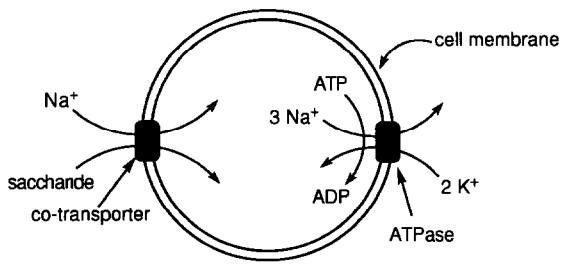
Marie-France Paugam, Gregory T. Morin, and Bradley D. Smith*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN, 46556, USA

Key Words: Active Transport, Glucopyranoside, Phenylboronic Acid, Ionophore, Liquid Organic Membrane

Abstract: *A carrier admixture of phenylboronic acid and ionophore (cryptand or crown ether) simultaneously co-transports p-nitrophenyl β-glucopyranoside, M^+ and OH^-, through a liquid organic membrane. The transport mechanism involves the apparent self-assembly of a lipophilic ion-pair, comprising of anionic, phenylboronate-glucopyranoside adduct and metal cation-ionophore complex. Active glucopyranoside transport (i.e. uphill transport against a concentration gradient) was achieved in the direction of a metal cation concentration gradient.*

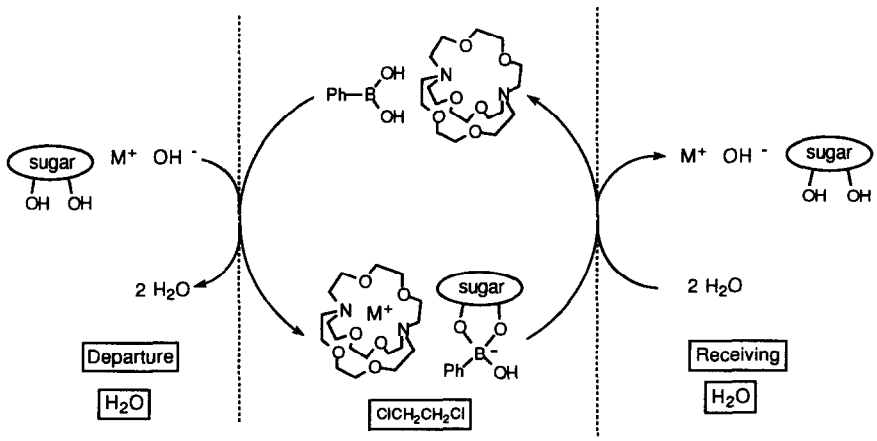
Facilitated transport of hydrophilic molecules across cell membranes is an important process in all living cells.[1] Two major pathways predominate; passive transport (facilitated diffusion) down a concentration gradient and active transport against a concentration gradient. A well-recognized example of secondary active transport, operating in prokaryotes and eukaryotes, utilizes membrane bound proteins to actively transport saccharide derivatives via a Na^+ co-transport (also known as symport) mechanism (Scheme 1).[1,2] An absolute requirement is the simultaneous translocation of both a saccharide and a Na^+ ion in the same direction. The typical intracellular Na^+ concentration is about fifteen times lower than extracellular,[3] therefore, the electrochemical $[Na^+]$ gradient drives saccharide transport actively into the cell. The low intracellular $[Na^+]$ is maintained by a Na^+-K^+ antiport which is driven by the hydrolysis of ATP via the enzyme Na^+,K^+-ATPase (Scheme 1). To our knowledge, an artificial metal cation/saccharide co-transport system capable of active saccharide transport driven by a metal cation gradient is unknown.[4] Such a system is the focus of this report.



Scheme 1. Na^+.Saccharide Co-transporter

Boronic acids are known to mediate the transport of highly water-soluble saccharide compounds through organic phases. Studies by Shinbo,[5] Czarnik,[6,7] and our group,[8] have shown that in the presence of tetraalkyl-ammonium cations, arylboronic acids can facilitate the transport of monosaccharides and ribonucleosides through bulk, liquid organic membranes. There is strong evidence that the transported species are lipophilic ion-pairs. The reversible complexation of polyols with boronic acids in aqueous solution to form covalent anionic,

tetrahedral boronate adducts is well known. The complexation is an acid-base equilibrium with the anionic, tetrahedral boronate adduct being favored in alkaline pH. Utilizing this fact, Shinbo was able to demonstrate active monosaccharide transport using a pH gradient as the electrochemical driving force.[5] Recently, we reported an example of active nucleoside transport mediated by phenylboronic acid (PBA) and driven by an anion gradient.[8] We now describe an alternative method of actively transporting saccharide derivatives mediated by PBA, this time utilizing a metal cation gradient as the energy source. Specifically, we have studied the transport of *p*-nitrophenyl β-glucopyranoside through a bulk, liquid organic membrane facilitated by a combined PBA/ionophore carrier system (Scheme 2). *p*-Nitrophenyl β-glucopyranoside was chosen as a model saccharide compound for two reasons; (i) Unlike reducing sugars, glycosides do not undergo mutarotation or ketose/aldose isomerization, thus speculation as to the nature of the saccharide portion of the transported species is less ambiguous,[9] (ii) The *p*-nitrophenyl β-glucopyranoside chromophore, which is stable under the conditions of the transport experiment, allowed ready determination of transport rates via UV absorption.



Scheme 2. Active Saccharide Transport Mediated by Phenylboronic Acid/[2.2.2]-Cryptand and Driven By Either M^+ or OH^- Ion Gradients.

The rates of *p*-nitrophenyl β-glucopyranoside transport through dichloroethane were determined by standard U tube experiments where an aqueous departure phase was separated from an aqueous receiving phase by a dichloroethane layer.[4,10] The passive transport experiments involved pre-equilibration of the carrier(s) between the three layers (if the carrier(s) remained only in the organic layer the concentration would have been 1 mM) followed by addition of the glucopyranoside to the departure phase (1.36 mM). After a short induction period the initial rates of appearance of glucopyranoside in the receiving phase were determined from the change in UV absorption (λ_{max} 302 nm, ε = 9,800 M^{-1} cm^{-1}) over a three hour period. As summarized in Table 1, a carrier combination of PBA and trioctylmethylammonium (TOMA) chloride (aqueous layers buffered at pH 11.0 with 10 mM sodium phosphate) produced a 16-fold enhancement in glucopyranoside transport over background, whereas PBA or TOMA alone had no effect.[11] Under identical conditions a PBA/[2.2.2]-cryptand carrier combination produced a 4-fold enhancement. Increasing the sodium phosphate concentration to 500 mM, raised the enhancement to 7-fold. Changing the buffer to 10 mM potassium phosphate resulted in a 10-fold transport enhancement, in agreement with the increased extraction of K^+ over Na^+ by [2.2.2]-

cryptand.[12,13] PBA in conjunction with the crown ether, dicyclohexyl-18-crown-6, (DCH18C6), produced only a slight enhancement in passive transport. The simplest explanation for the lower efficiency of the PBA/ionophore system compared to PBA/TOMA is the ionophore is not completely occupied with M^+ and/or the resulting cationic complex is not as lipophilic as TOMA. Thus, the effective concentration of lipophilic ion-pair kinetically capable of transport is lower.

Table 1. Rates of Passive Glucopyranoside Transport.

Carrier	Rate[a] /nM min^{-1} (% Transported in 3 hours)
PBA/TOMA	453 (9.5)[b]
PBA/[2.2.2]	113 (2.4),[b] 184 (3.8),[c] 217 (4.6)[d]
PBA/DCH18C6	38 (0.7)[b,d]
[2.2.2]	20 (0.4)[b]
PBA	28 (0.6)[b]
TOMA	30 (0.6)[b]
none	20 (0.4)[b]

[a]Rate Constants are ± 20 % Both aqueous layers are; [b]10 mM sodium phosphate, pH 11 0 [c]500 mM sodium phosphate, pH 11.0 [d]10 mM potassium phosphate, pH 11.0

The transport cycle described in Scheme 2 is formally a glucopyranoside, M^+, and OH^- symport mechanism. It predicts that uphill pyranoside transport can be driven in the direction of a $[M^+]$ and/or $[OH^-]$ gradient. Active transport experiments driven by a $[M^+]$ gradient were conducted using carrier concentrations of [PBA] = 1 mM and [cryptand] = 3 mM. The experiments began with equal concentrations of glucopyranoside (0.06 mM) in each aqueous phase buffered at pH 10.0 with sodium carbonate. The buffer concentrations were 1 M in the departure phase and10 mM in the receiving phase. Uphill glucopyranoside transport in the direction of the $[Na^+]$ gradient was achieved using PBA/[2.2.2]-cryptand as described in Figures 1 and 2. At the end of the run the pH in both aqueous phases was found to be unchanged, hence a pH difference was not the cause of the uphill transport.[14] As well, transport due to a difference in ionic strengths was ruled out because control experiments with the following carrier combinations all failed to produce $[Na^+]$-driven active transport; PBA/trioctylamine (Figure 2), PBA/TOMA (Figure 2), PBA alone, and [2.2.2]-cryptand alone. Taken together, these results confirm the M^+/glucopyranoside symport mechanism of Scheme 2. When the active transport experiment with PBA/[2.2.2]-cryptand was repeated with potassium carbonate as the buffer, the transport rate was observed to be slightly lower (Figure 2). According to Scheme 2, active saccharide transport is only achieved when an M^+ ion moves from the organic layer into the receiving phase along with OH^- and a saccharide molecule. Such a mechanism predicts that the rate of active saccharide transport can be no faster than the rate of M^+ transport.[13] It is known from the literature that although [2.2.2]-cryptand extracts K^+ more effectively than Na^+, it transports K^+ much slower than Na^+ due to the slow, rate-limiting release of K^+ into the receiving phase.[12] Thus the slower rate of $[K^+]$-driven active saccharide transport most likely reflects the slower rate of K^+ transport.

The PBA/ionophore carrier system described in Scheme 2 represents an artificial but functionally biomimetic M^+/saccharide co-transport system. It suggests a way of utilizing a ubiquitous, biotic energy source (the transmembrane $[Na^+]$ gradient) to not only transport but concentrate hydrophilic compounds (e.g. drugs) inside a cell. Improvements in the carrier design can be envisioned,[15] and are the focus of current studies.

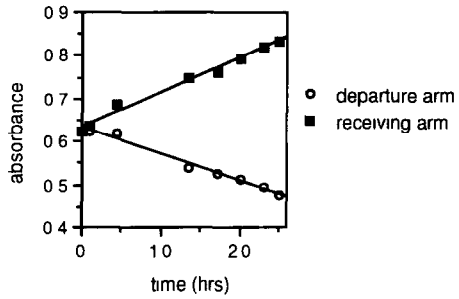


Figure 1. Change in *p*-nitrophenyl β-glucopyranoside absorbance (302 nm) in the departure (1 M sodium carbonate) and receiving (10 mM sodium carbonate) arms, pH 10.0, carrier system PBA/[2.2.2]-cryptand

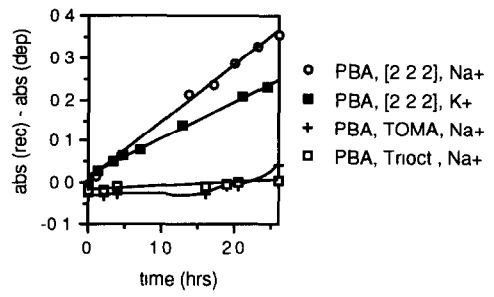


Figure 2. Difference between *p*-nitrophenyl β-glucopyranoside absorbance (302 nm) in the departure (1 M carbonate) and receiving (10 mM carbonate) arms, pH 10 0

Acknowledgment: This work was supported by a grant from the National Science Foundation (CHE 93-11584). G.T.M acknowledges the University of Notre Dame for a Nieuwland Fellowship, and M.-F.P is grateful for a Lubrizol Fellowship.

REFERENCES AND NOTES

1. Stein, W. D., *Channels Carriers and Pumps, An Introduction to Membrane Transport*, Academic: San Diego, 1990.
2. (a) Wright, J. K.; Seckler, R.; Overath, P, *Ann. Rev. Biochem* , **1986**, *55*, 225-248. (b) Nikaido, H.; Saier, M. H., *Science*, **1992**, *258*, 936-942.(c) Yamamato, I.; Anraku, Y. in *Alkali Cation Transport in Prokaryotes*, Bakker, E. P., Ed.; CRC Press: New York, 1993, Ch. 1C.
3. Alberts, B.; Bray, D.; Lewis, J.; Raff, M.; Roberts, K ; Watson, J. D. *Molecular Biology of the Cell*, Garland: New York, 1989, p. 301.
4. *Liquid Membranes. Chemical Applications*, Araki, T., Tsukube, H., Eds ; CRC Press:Boca Raton, 1990.
5. Shinbo, T.; Nishimura, K.; Yamaguchi, T ; Sugiura, M., *J Chem Soc , Chem Commun.*, **1986**, 349-351.
6. Grotjohn, B. F.; Czarnick, A. W., *Tetrahedron Lett.*, **1989**, *30*, 2325-2328. Mohler, L. K.; Czarnik, A W. *J. Am Chem. Soc* , **1993**, *115*, 2998-2999.
7. Czarnik has shown that amino acids are also transported by PBA, although the transport mechanism is slightly different to that described here. Mohler, L K.; Czarnik, A W., *J Am Chem Soc.*, **1993**, *115*, 7037-7038.
8. Paugam, M. -F.; Smith, B. D *Tetrahedron Lett.*, **1993**, *34*, 3723-3726.
9. (a) Ferrier, R. J., *Adv Carbohydr Chem.* **1978**, *35*, 31-80. (b) Chapelle, S.; Verhere, J.-F *Tetrahedron*, **1988**, *44*, 4469-4482.
10. The apparatus and method have been described in Ref. 8. All transport runs were reproduced at least in duplicate.
11. The transported glucopyranoside species is most likely the anionic 4:6-phenylboronate adduct. Gorin, P. A. J.; Mazurek, M. *Carbohydr Res* , **1973**, *27*, 325-339. Gorin, P A. J., Mazurek, M *Can J Chem* , **1973**, *51*, 3277-3286
12. Lamb, J. D.; Christensen, J. J., Oscarson, J. L., Nielsen, B. L.; Asay, B. W.; Izatt, R. M., *J Am Chem. Soc* , **1980**, *102*, 6820-6824.
13. In addition to the mechanism described in Scheme 2, passive transport can also occur via a second pathway where the cryptand/M^+ complex does not disassociate but instead saccharide and OH^- are simply exchanged at the organic/receiving interface for a Cl^- (see the mechanism described in Ref. 8) The transport rate for this pathway is predicted to increase with the extractability of the cryptand/M^+ complex, and be independent of the rate of cryptand/M^+ dissociation. Hence, passive transport mediated by [2.2.2]-cryptand/K^+ is observed to be faster than [2.2.2]-cryptand/Na^+. As explained in the text, the reverse trend is observed for active transport.
14. At the end of some runs the pH levels in one or both aqueous phases had dropped slightly. Control experiments clearly showed that the observed active transport was not due to these small pH differences For example, starting a run with the departure phase at pH 10.0 and the receiving phase at pH 10.5 still produced significant active transport in the direction of the [M^+] gradient and against the pH gradient.
15. (a) Reetz, M T.; Niemeyer, C. M ; Harms, K., *Angew Chem., Int. Ed. Engl* , **1991**, *30*, 1472-5. (b) Williams, G.; Tuladhar, S. M.; D'Silva, C., *J. Phys. Org Chem* , **1992**, *5*, 437-442

(Received in USA 13 August 1993; accepted 29 September 1993)