Boronic Acids Selectively Facilitate Glucose Transport through a Lipid Bilayer†

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Glucose is the most important carbohydrate in human metabolism. Many body tissues depend on glucose as a primary source of energy. To ensure that a sufficient supply is always available, the blood glucose concentration is maintained around 5 mM. In addition, the tissues retain an abundance of glucose transporters to facilitate glucose movement across the cell membranes.[1] The transporters are integral proteins that act as passive and/or active membrane transport systems. Currently, there is much interest in elucidating the mechanism of these biotic transporters. While much structural data has been accumulated, the kinetic picture is still largely unknown.[2] The development of artificial transporters for carbohydrate compounds, such as glucose, is a complementary research goal. In terms of applied technology, artificial transporters have potential as reagents for modulating the membrane permeability of selective biochemicals, or as drug transport devices for improving therapeutic efficacy. From the perspective of basic research, artificial systems represent simplified models that allow some of the structural and kinetic aspects of membrane transport theory to be tested.[3] Previously, we and others have investigated the ability of boronic acids to facilitate the transport of saccharide derivatives through liquid organic membranes.[4] Herein, we report that simple arylboronic acids, **1–7**, and the alkyl derivative **9** are able to selectively facilitate the efflux of glucose from liposomes. To our knowledge this represents the first example of selective transport of a carbohydrate compound through a lipid bilayer mediated by an abiotic carrier.

Glucose (typically 300 mM) was encapsulated inside large unilamellar vesicles (LUVs, 80 nm diameter, encapsulation volume 1.1 μL/μmol of lipid), composed of dipalmitoylphosphatidyl choline (DPCC), cholesterol (C), and phosphatidic acid (PA) in the ratio 20:15:2. The liposomes were prepared by the rapid extrusion technique and were found to be essentially impermeable to glucose leakage over a number of days.[5,6] Figure 1 describes the glucose efflux experiment, which uses the standard hexokinase/glucose-6-phosphate dehydrogenase enzyme system for detection of escaped glucose.[7] The enzymes are unable to penetrate the liposomes, thus an absorbance reading at 340 nm,

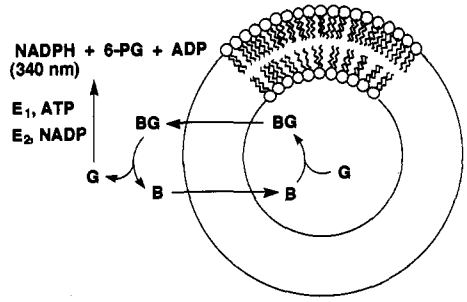

Figure 1. Liposome glucose efflux experiment. G = glucose, B = boronic acid, BG = glucose–boronate complex, E_1 = hexokinase, E_2 = glucose-6-phosphate dehydrogenase, 6-PG = 6-phosphogluconate, NADP = nicotinamide adenine dinucleotide phosphate, NADPH = reduced form of nicotinamide adenine dinucleotide phosphate, ADP = adenosine diphosphate, ATP = adenosine triphosphate.

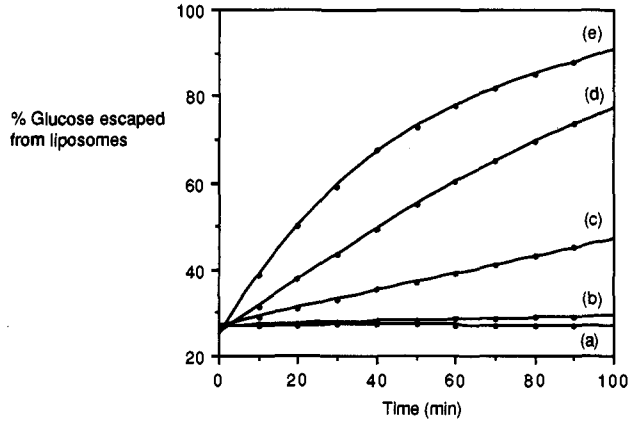

Figure 2. Percent glucose escaped at pH 7.5, from liposomes (LUVs; diameter 80 nm; composition, 20:15:2 DPPC:C:PA; total lipid concentration, 0.75 mM) containing 300 mM glucose, after treatment with (a) no boronic acid; (b) **1**, 1 mM; (c) **1**, 5 mM; (d) **2**, 1 mM; and (e) **3**, 1 mM.

due to NADPH formation, results only when a glucose molecule is released from the liposome. This proved to be a satisfactory assay system because the rates of glucose efflux were generally slow compared to the kinetics of the enzyme assay.[8] As shown in Figure 2, addition of various boronic acid compounds induced glucose leakage from the liposomes. The rates of glucose efflux exhibited an approximate first-order dependence on boronic acid concentration.[8] Glucose efflux from the liposomes continued until the liposomes were completely empty. This active transport effect is attributed to the destructive assay which continually removes glucose from the system.

The following control experiments strongly indicated that the glucose efflux was due to a selective transport process and not a general increase in liposome permeability.

1. Essentially no glucose efflux was observed upon addition of large amounts of structurally related organic compounds such as phenol or benzoic acid derivatives (100 mM), or organic solvents such as methanol and dimethyl sulfoxide (up to 10% of the sample volume). As described in Table 1, negligible glucose efflux was

† Molecular Recognition with Boron Acids. 6. Part 5: See ref 4a.

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(6) The liposomes were prepared according to the method of MacDonald, R. C.; MacDonald, R. I.; Menco, B. P. M.; Takeshita, K.; Subbarao, N. K.; Hu, L. *Biochim. Biophys. Acta* **1991**, *1061*, 297–303. A detailed procedure is described in the supplementary material.

(7) Kinsky, S. C. In *Methods in Enzymology*; Fleischer, S., Packer, L., Eds.; Academic Press: London, 1974; Vol. 32, pp 501–512. Details of the glucose efflux assay are provided in the supplementary material.

(8) Glucose-6-phosphate dehydrogenase was found to be inhibited by lipophilic anions. Because of this, the glucose efflux assay became less reliable as the concentration of boronate anion increased. In particular, when high concentrations of the more acidic boronic acids were used, the enzyme assay became rate-determining and the increase in absorbance at 340 nm was slower than the rate of glucose efflux.

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Table 1. Relative Rates of Glucose Efflux at pH 7.5, pK_a's, and Substituent Hydrophobicity Constants for Various Boronic Acid Derivatives

boronic acid, $RB(OH)_2$	rel rate of efflux (±15%)	pK_a	sum of π values[a]
phenylboronic acid, **1**	1	8.9[b]	0.0
(3,5-dichlorophenyl)boronic acid, **2**	15	7.4[c]	1.42
[3,5-bis(trifluoromethyl)phenyl]boronic acid, **3**	30	7.2[c]	1.76
(4-methylphenyl)boronic acid, **4**	1.9	9.3[b]	0.56
(4-methoxyphenyl)boronic acid, **5**	0.8	9.3[b]	–0.02
(3-methoxyphenyl)boronic acid, **6**	0.9	8.7[c]	–0.02
(4-*tert*-butylphenyl)boronic acid, **7**	30	9.3[c]	1.98
(4-carboxyphenyl)boronic acid, **8**	0.02	8.4[d]	–4.36
1-butylboronic acid, **9**	0.3	10.4[e]	
boric acid, **10**	10^{-4}	9.0[c]	

[a] Sum of the hydrophobicity constants for all aryl substituents except the boronic acid, ref 9a. [b] Reference 9b. [c] Determined by the method described in ref 9c. [d] Reference 9d. [e] Reference 9e.

observed in the presence of extremely hydrophilic boron acids such as (4-carboxyphenyl)boronic acid, **8** (25 mM), and boric acid, **10** (100 mM).

2. Experiments with other encapsulated marker compounds showed no change in liposome permeability under the conditions used to determine glucose efflux. For example, a standard leakage experiment with carboxyfluorescein was conducted.[10] When encapsulated at a high concentration, carboxyfluorescein produces little fluorescence due to self-quenching; however, once carboxyfluorescein is released from the liposomes, fluorescence increases significantly. This phenomenon was immediately observed when the liposomes were lysed with the detergent Triton X-100, but no such efflux was observed when the liposomes were treated with any of the boronic acids described in Figure 2. Similar experiments with the dyes, calcein (fluorescence assay),[10] and arsenazo III (absorption assay),[11] as well as the anionic carbohydrate derivatives, glucose 6-phosphate and isocitrate (dehydrogenase enzyme assays),[12] gave the same results.[13]

3. Leakage experiments were conducted with a mixture of glucose (200 mM) and calcein (100 mM) encapsulated inside the liposomes. Treatment with the boronic acids described in Figure 2 produced glucose efflux rates very similar to those observed in the absence of encapsulated calcein. The fluorescence assay, however, showed no calcein leakage. Thus the boronic acids were able to selectively transport glucose in the presence of calcein.

Attempts to elucidate the transport mechanism have been initiated. As with any liposome efflux experiment the possibility of a membrane potential complicates any mechanistic interpretation. Previous work with liquid organic membranes has shown that boronic acids can transport diol compounds by forming reversible trigonal or tetrahedral diol–boronate complexes.[4] Inspection of Table 1 suggests that efflux is more dependent on the lipophilicity of the boronic acid (as judged by hydrophobicity constant, π) than the acidity (as judged by pK_a).[8,14] To gain further mechanistic insight, the effect of pH and added lipophilic ions on glucose efflux rates was investigated. The direction of the pH effect depended on the pK_a of the boronic acid; glucose efflux was a maximum when the pH was just below the boronic acid pK_a. The effect of added lipophilic ions was independent of boronic acid identity. In the presence of tetrabutylammonium chloride (5 mM), boronic acid mediated efflux increased 2–4 fold. Addition of large amounts of sodium perchlorate (150 mM), however, had no effect on efflux rates. Control experiments showed that, in the absence of boronic acid, liposome permeability was unaffected by these changes in pH or salt additions. Repeating the glucose efflux experiments with positively charged liposomes (*i.e.*, liposomes composed of DPPC:C:dodecyltrimethylammonium bromide, 20:15:2), under the conditions described in Figure 2, resulted in very similar rates of glucose efflux.

With the evidence in hand, it appears that the transport mechanism includes the following key points. The neutral boronic acid enters the liposome and combines with an appropriate diol functionality on the glucose to form a tetrahedral glucose–boronate anion that is attracted to the ionic bilayer surface. In the presence of added lipophilic cations, the tetrahedral glucose–boronate may traverse the bilayer as a lipophilic ion pair. Whether a counterion is present when the lipophilic cations are absent is not clear. A counter-transport mechanism involving entry of uncomplexed boronate anion into the liposome with concomitant exit of anionic glucose–boronate complex is plausible. On the other hand, the tetrahedral glucose–boronate may be in equilibrium with its neutral, conjugate-acid, trigonal structure, which is the actual transported species. The fact that weak organic acids and bases can permeate bilayer membranes via their chemically neutral forms (even under conditions where the neutral forms are present in minor amounts) is evidence in favor of the latter explanation.[15] Additional mechanistic studies are in progress, as well as efforts to ascertain if other biomolecules can be transported by this method.[13]

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Supplementary Material Available: Detailed procedures for boronic acid syntheses, all efflux experiments, and representative sets of raw efflux data (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(11) Reference 5, pp 129–131.

(12) The use of isocitrate dehydrogenase as an assay for isocitrate efflux has been reported before. Crowe, L. M.; Womersley, C.; Crowe, J. H.; Reid, D.; Appel, L.; Rudolph, A. *Biochim. Biophys. Acta* **1986**, *861*, 131–140.

(13) Since all the control leakage experiments used anionic compounds, there remains a possibility that the boronic acids perturb the liposomes in such a way that neutral compounds can escape, but charged ones are retained. Our most recent results appear to rule out this possibility. We have observed that boronic acids greatly facilitate the efflux of ribonucleosides but do not transport arabinonucleosides, in agreement with the known selectivity of boronic acids for cis vicinal diols.

(14) The dependence on boronic acid lipophilicity is most clearly demonstrated by comparing the efflux rates induced by **4**, **5**, and **7**. These compounds have virtually identical p*K*a's but different π values (Table 1).

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