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SELECTIVE TRANSPORT OF RIBONUCLEOSIDES THROUGH A LIQUID MEMBRANE[1]

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Summary: Lipophilic salts of phenylboronic acid facilitate the transport of ribonucleosides across a $ClCH_2CH_2Cl$ liquid membrane; deoxynucleosides, in general, are not transported in this system.

Facilitated transport of highly water-soluble species through hydrophobic cell membranes is an essential biochemical process. While elegant work has been reported modelling the transport of ionic species (e.g., metal ions[2] and nucleotides[3]) through liquid membranes in "U-tube" type experiments, little work has been described on the equally relevant transport of neutral compounds. For example, sugar transport in gram-positive bacteria has been well documented[4] and a model transport system has been reported by Shinbo.[5] In human erythrocytes the relevant transport protein effects delivery of adenosine at about 20-times the rate due to simple diffusion. Inasmuch as adenosine at high concentration serves as a myocardial depressant, adenosine is a primary regulator of blood flow to the heart.[6] Recently, transport of adenine, adenosine, and deoxyadenosine in a model system has been described utilizing a synthetic receptor capable of hydrogen bonding to the adenine ring system.[7] We now report that ribonucleosides are transported through a liquid membrane by reversible complexation with phenylboronic acid salts. Furthermore, the anticipated recognition of the vicinal diol group achieves a selective transport of ribonucleosides as compared to deoxyribonucleosides, by factors as high as 200-fold.

To measure the rates of unfacilitated nucleoside transport through a dichloroethane liquid membrane, U-tubes (11.5 mm i.d.) were charged by adding first dichloroethane (7 mL), followed by pH 7.5 HEPES buffer (0.1 M; 3.5 mL) to both arms (designated α and β). The dichloroethane layer was stirred using a "flea" stirbar and an electromagnetic stirrer capable of delivering a constant 300 rpm to all experiments;[8] this stir rate was insufficient to generate mechanical transfer of aqueous solution from one arm to the other. At time=0, a concentrated solution of nucleoside was added to the α-arm sufficient to provide an initial concentration of 17 μM in the α-arm. The change in absorbance of the β-arm was then monitored by UV as a function of time. Facilitated transport was determined using an identical experimental protocol, but in this case the dichloroethane and HEPES buffer used were stirred

together vigorously with sufficient added phenylboronic acid ("PBA") and trioctylmethylammonium bromide ("TOMA") to give a final concentration of 10 μM overall. Under these conditions, UV indicates that the phenylboronic acid salt partitions between the organic (75%) and aqueous (25%) layers.

A typical set of kinetic results are shown in Figure 1. The figure shows the raw data from four experiments: transport of uridine with (▲) and without (Δ) added PBA-TOMA, and transport of deoxyuridine with (•) and without (○) added PBA-TOMA. Both experiments with added PBA-TOMA have an initial absorbance of ca. 0.2 because PBA absorbs at the wavelength of interest (264 nm). The unfacilitated transport of deoxyuridine is faster than that of uridine, consistent with the expected lower polarity of 2'-deoxyuridine compared to uridine; this finding was general for all the nucleosides examined (Table 1). **However, upon introduction of 10 μM PBA-TOMA, this selectivity reverses for all nucleosides.** After an induction period (due likely to a lack of agitation near the α-arm interface) the facilitated transport of uridine increases to a final rate of 430-times that of the unfacilitated rate. By comparison, the facilitated transport of deoxyuri-

FIGURE 1

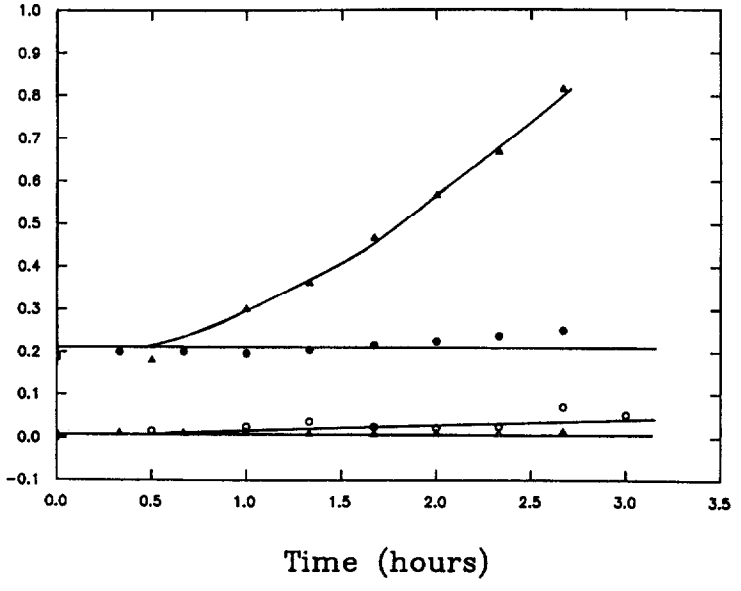


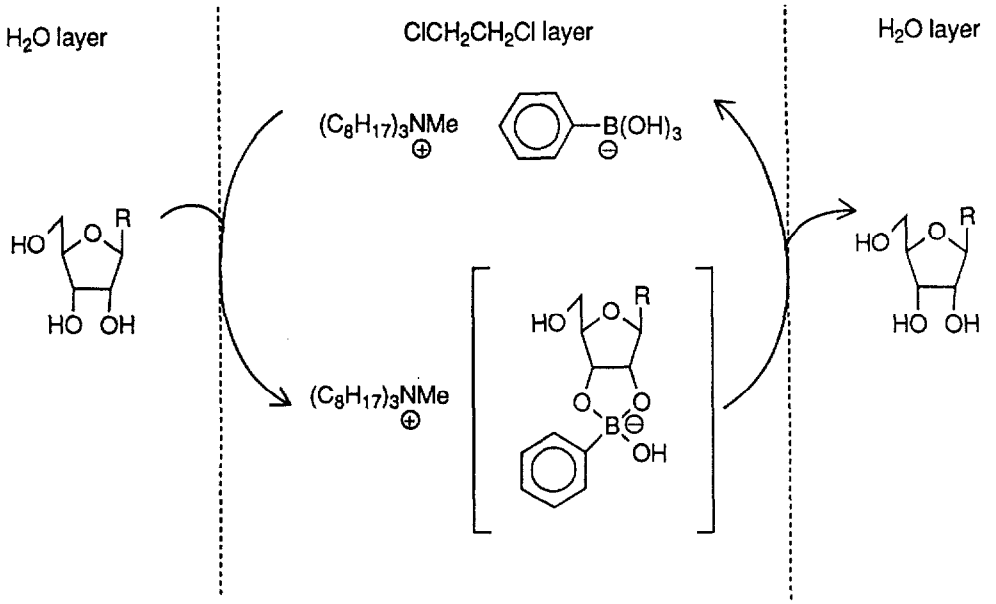
Table 1. Relative Transport Rates of Ribo- and Deoxyribonucleosides.

Compound	Rate without added carrier	Rate with added carrier	Rate ratio	λ (nm)
adenosine	.011	.48	44	262
deoxyadenosine	.113	.073	.65	262
inosine	.002	.52	260	252
deoxyinosine	.005	.19	38	250
uridine	.0008	.34	430	264
deoxyuridine	.013	.027	2.1	264
cytidine	.002	.017	8.5	272
deoxycytidine	.026	.006	.23	270

Due to the differing extinction coefficients of these nucleosides, only rates within a series (e.g., adenosine and deoxyadenosine) can be compared directly. Relative rates are based on at least two independent runs; rates for adenosine and deoxyadenosine are based on 6 independent runs. All relative rates are reported ±20%.

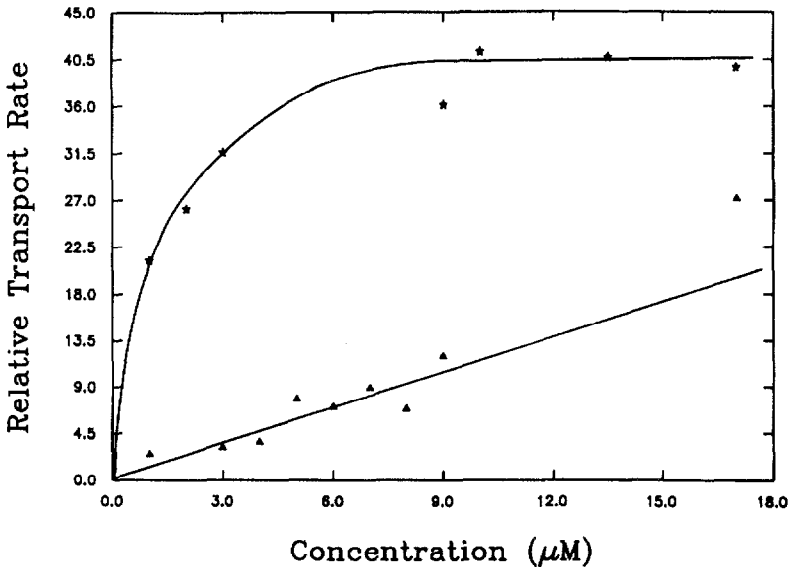
dine is comparatively unchanged (2.1-fold increase) by addition of PBA-TOMA.[9] This observation strongly suggests that the mode of transport by PBA involves reversible formation of a cyclic boronate complex with the 2',3'-diol group in ribonucleosides (Figure 2). Such complexation with ribonucleosides is, of course, well precedented, as is the absence of complexation with deoxyribonucleosides.[10]

FIGURE 2



Facilitated transport by the PBA-TOMA carrier revealed saturation behavior, consistent with the proposed chemical mechanism of action. The relative rates for adenosine transport, calculated after the induction period, were determined as a function of the carrier concentration as shown in Figure 3. Increasing the amount of carrier increased the rate of transport to a maximum level (44-fold acceleration), after which no additional rate increase was observed (*). In

FIGURE 3



addition, increasing the amount of adenosine in the α-arm at constant (1 μM) carrier concentration yielded a roughly linear increase in transport rate (▲). Neither the transport of adenosine nor of deoxyadenosine were accelerated by addition of only PBA or of only TOMA to the system, suggesting that the boronate complex-TOMA ion pair indicated in Figure 2 is the kinetically competent species responsible for ribonucleoside transport.

In summary, we have shown for the first time that the selectivity of ribo- vs. deoxyribonucleoside transport through a bulk liquid membrane may be reversed by introduction of a simple carrier molecule that models the action of naturally occurring transport proteins. While carbohydrate binding proteins likely accomplish the complexation of neutral sugars by methods other than reversible covalent bond formation,[11] the boronate approach offers an accessible entry into the design of totally synthetic carbohydrate receptors. As such, the boronate approach to vicinal diol complexation appears highly complementary to the hydrogen-bonding/pi stacking approach to nucleic acid base complexation, leading ultimately to the construction of more sophisticated ribonucleoside binding molecules. Efforts directed towards the improvement of boronate-mediated ribonucleoside transport may be envisioned, and are the focus of ongoing efforts in this group.[12]

References and Notes

1) Presented at the Fifth International Symposium on Inclusion Phenomena and Molecular Recognition, Orange Beach, Alabama (Sept 88).

2) For an overview, see the 1987 Nobel lecture by D. J. Cram: Cram, D. J. *Journal of Inclusion Phenomena* **1988**, *6*, 397.

3) For an overview, see the 1987 Nobel lecture by J.-M. Lehn: Lehn, J.-M. *Journal of Inclusion Phenomena* **1988**, *6*, 351.

4) For an overview, see: "Sugar Transport and Metabolism in Gram-Positive Bacteria"; Reizer, J. and Peterkofsky, A., eds.; Wiley, NY, NY; 1987.

5) Shinbo, T.; Nishimura, K.; Yamaguchi, T; Sugiura, M., *J. Chem. Soc. Chem. Commun.*, **1986**, *349*.

6) Fox, I. H.; Kelley, W. N. *Ann. Rev. Biochem.* **1978**, *47*, 655.

7) Benzing, T.; Tjivikua, T.; Wolfe, J.; Rebek, J. *Science* **1988**, *242*, 266.

8) This Variomag Electronicuhrer Multipoint HP15 stirring plate was purchased from the Fisher Scientific Company, Pittsburgh, PA.

9) In the deoxy- series, only the transport of deoxyinosine is significantly accelerated by the 10 μM PBA-TOMA carrier system (38-fold). A possible explanation for this unexpected observation is PBA complexation involving both the 5'-hydroxyl group and N-3 of deoxyinosine. Of the four deoxynucleosides examined, only deoxyinosine has an enolizable proton on a ring nitrogen geometrically disposed for boronate formation. The formation of amine-containing boronate complexes is precedented: (a) Philipp, M.; Bender, M. L. *Proc. Nat. Acad. Sci.* **1971**, *68(2)*, 478; (b) Bachovchin, W. M. *Biochemistry*, **1988**, *27*, 7689.

10) Ferrier, R. J. *Adv. Carb. Chem. Biochem.* **1978**, *35*, 31.

11) A recent example may be found in the X-ray structure of the sugar binding site of the E. coli galactose chemoreceptor protein: Vyas, N. K.; Vyas, M. N.; Quiocho, F. A. *Science* **1988**, *242*, 1217.

12) We thank the American Cancer Society for Support of this work.

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