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Active Transport of Uridine Through a Liquid Organic Membrane Mediated by Phenylboronic Acid and Driven by a Fluoride Ion Gradient

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Abstract: *Phenylboronic acid simultaneously co-transports the ribonucleoside, uridine, and fluoride ion through an organic liquid membrane. The transport mechanism involves self-assembly of a lipophilic ion-pair comprising of phenylboronic acid, fluoride ion, uridine and tetralkylammonium cation. Active uridine transport (i.e. uphill transport against a concentration gradient) occurs in the direction of a fluoride ion concentration gradient.*

Although there have been numerous reports of abiotic transport systems capable of transporting neutral, hydrophilic ligands through organic liquid membranes, examples of systems that can transport such ligands actively (i.e. uphill against a concentration gradient) are quite rare.[1,2] Phenylboronic acid (PBA) is known to facilitate the transport of saccharides and ribonucleosides through organic liquid membranes.[3-5] These water soluble, diol-containing ligands react reversibly with the PBA to form lipophilic adducts. The evidence indicates these adducts are anionic, tetrahedral boronate complexes and the transported species are in fact lipophilic ion-pairs. Formation of the anionic boronate is favored at alkaline pH and disfavored at lower pH. Utilizing this fact, Shinbo was able to demonstrate active saccharide transport using a pH gradient as the electrochemical energy source.[3] Recently, Reetz *et al.* showed that F^- ions can form dative bonds to trigonal boronic acid esters in organic solvents to produce tetrahedral fluoroboronate anions.[6] This result prompted us to examine the possibility of actively transporting hydrophilic, diol-containing ligands through liquid organic membranes using PBA as the carrier and a F^- ion concentration gradient as the electrochemical driving force. Here we report the passive and active transport of the ribonucleoside, uridine, which apparently involves the self-assembly of a lipophilic ion-pair comprising of PBA, F^- ion, uridine and tetralkylammonium cation.

Uridine transport was measured via standard U tube experiments where an organic layer (1,2-dichloroethane, 7.0 mL) separated an aqueous departure phase (0.5 M HEPES, pH 7.0, 3.0 mL) in one arm of the U tube (i.d. 12 mm) from an identical aqueous receiving phase in the other arm.[1,7] First we examined the effect of F^- ions on passive uridine transport. To the organic layer was added PBA (1 mM) and trioctylmethylammonium chloride (TOMAC) (1 mM) and the system allowed to equilibrate by stirring for five hours (after equilibration approximately 25% of the PBA had partitioned into the aqueous layer[4]). Uridine (5 mM) was then added to the departure arm and its rate of appearance in the receiving arm was determined by UV absorption at 261 nm. After an induction period, the uridine was transported at a constant rate (5 nM s^{-1}) in agreement with Grotjohn and Czarnik.[4] Repeating the experiment with KF (0.5 M) added to the departure arm produced an approximate

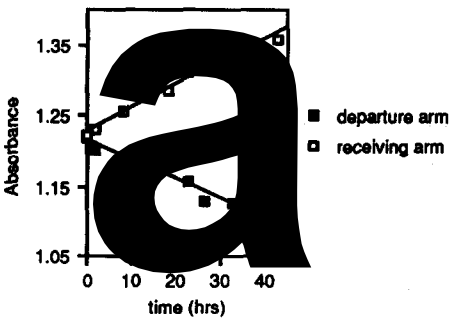


Figure 1. Change in uridine absorbance (261 nm) in the departure and receiving arms upon addition of KF to the departure arm, pH = 7.0.

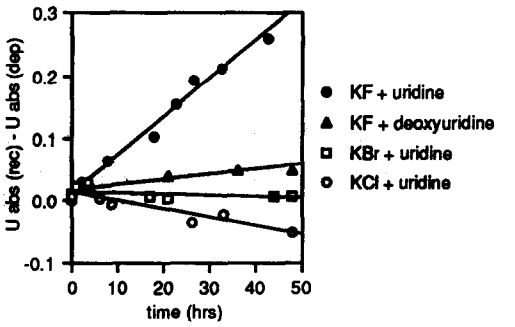
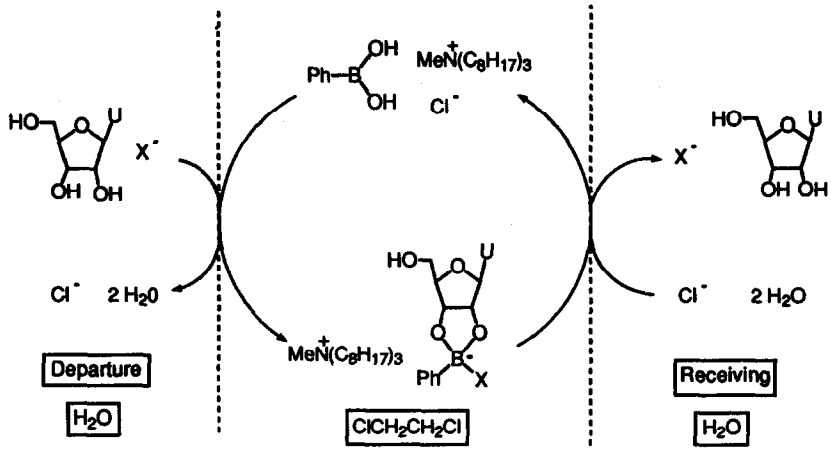


Figure 2. Difference between uridine (U) absorbance (261 nm) in the departure and receiving arms upon addition of KX to the departure arm, pH = 7.0.

three-fold increase in the rate of uridine transport, whereas adding an equivalent amount of KBr had no effect on the transport rate. The active transport experiments started with equal uridine concentrations (0.125 mM) in each arm, and PBA (1 mM) and TOMAC (1 mM) in the organic layer. The layers were allowed to fully equilibrate before KX (0.1 M) was added to the departure arm. After a second short equilibration time, the concentration of uridine in each arm was monitored by UV absorption. As shown in Figures 1 and 2, when X = F the uridine concentration progressively decreased in the departure arm and increased in the receiving arm.[7] Under the same conditions, negligible active transport of 2'-deoxyuridine was observed (Figure 2) confirming that a *cis*-diol configuration on the ligand is required for complexation.[4] When X = Cl, a low rate of active uridine transport in the direction counter to the Cl^- gradient was observed. In the case of X = Br there was no apparent change in uridine concentrations.

All these observations are well rationalized by the transport cycle described in Scheme 1. In the absence of F^-, passive uridine transport is mediated by the reversible formation of the cyclic, anionic boronate adduct where X = OH, as described by previous workers.[3,4] When both arms of the U tube have equal uridine concentrations and the same pH, no active transport is observed as there is no electrochemical driving force. In the presence of F^- ions a second transport pathway is possible via the uridine phenylfluoroboronate adduct formed where X = F in Scheme 1. The PBA acts as a carrier for the simultaneous co-transport of uridine and F^- ion; to preserve neutrality Cl^- ion is transported in the reverse direction. The passive uridine transport experiments showed an enhanced transport rate upon addition of F^- ions to the departure arm due to the participation of this extra pathway. In the case of the active transport experiments, addition of F^- ions to the departure arm drives uridine transport uphill towards the receiving arm. Examination of Scheme 1 also explains the apparent reverse active transport observed in the case of X = Cl and the zero transport observed when X = Br. Under the conditions of the transport experiments, only dative bonding by X = OH and F is significant, dative bonding by X = Cl or Br is negligible. Thus the observed effect of the added Cl- and Br- ions is due only to their electrochemical gradients. The added Cl^- ions spontaneously move downhill from the departure arm towards the receiving arm. To preserve neutrality, tetrahedral PBA anions either complexed and/or not complexed with uridine, are forced to transport in the reverse direction. In the case of X = Br, the added Br^- ions moving towards the receiving arm can be replaced by Cl^- ions from the TOMAC, hence no change in absorbance is observed.



Scheme 1. Uridine Transport Mediated by PBA and X = F or OH.

Evidence in favor of the proposed stabilities of the putative tetrahedral phenylhaloboronate adducts was gained by examining the complexation of PBA ethylene glycol ester, $PhB(O_2C_2H_4)$, with NBu_4X salts (X = F, Cl, Br) in $CDCl_3$ using multinuclear NMR spectroscopy. Only in the case of X=F was complexation observed to produce the $PhB(O_2C_2H_4)F^-$ adduct.[8] Evidence that F^- ions can also complex with PBA derivatives in water to form tetrahedral phenylfluoroboronates was gained from UV titration experiments.[9] Trigonal PBA in acidic solution absorbs at λ_{max} = 218 nm. Titration with KOH produces the tetrahedral $PhB(OH)_3^-$ anion (pKa of PBA = 8.86[10]) which absorbs at λ_{max} = 204 nm. Titration of a PBA solution, buffered at pH 3, with KF also showed conversion to a spectrum absorbing at λ_{max} = 204 nm . The most likely explanation for this transformation is reaction of F^- with the PBA to form phenylfluoroboronate anions, $[PhB(OH)_nF_{3-n}]^-$, n = 0 - 2. The equivalence point for the F^- mediated conversion of trigonal to tetrahedral phenylboronate species occurred at 15 mM KF (Figure 3) and was basically unchanged when the titration was repeated in the presence of excess 1-methoxy-2,3-propanediol.

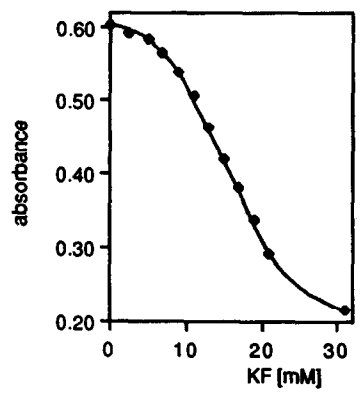


Figure 3. PBA absorbance at 218 nm as a function of [KF], pH = 3.0.

In conclusion, we report the first abiotic example of active transport of a neutral, hydrophilic ligand through a lipophilic membrane driven by an electrochemical gradient other than pH. The transport system utilizes a symport mechanism involving the co-transport of a diol-containing ligand and F^- ion in the same direction, mediated by a boronic acid carrier. A potentially useful application of this finding is in the area of boronate affinity chromatography. This commonly used purification technique utilizes boronic acids immobilized on solid supports for the separation of a range of biomolecules including nucleosides, nucleotides, saccharides and glycosylated proteins.[11] In general, maximal binding to the commercially available boronate matrices is achieved using conditions of pH 8.5 or above and high ionic strength. Since many biological samples are unstable under these conditions, the ability to strongly bind ligands at neutral pH and low ionic strength would be very useful. The results reported here demonstrate that nucleoside/boronic acid complexation at pH 7 is significantly enhanced by the presence of F^- ions. We are currently examining if F^- ions also improve the binding of nucleosides and nucleotides to immobilized boronic acids.

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7. The organic phase in the middle of the U tube was magnetically stirred at a rate that avoided mechanical mixing of the layers. The pH levels of the aqueous layers in each arm were unchanged by the KF addition. Control experiments showed that in the absence of PBA or TOMAC, the added KF was unable to facilitate uridine transport. All transport experiments were reproduced at least in duplicate.
8. $PhB(O_2C_2H_4)$, ^{13}C NMR 134.7 (ortho), 131.4 (para), 127.7 (meta), 65.9 (CH_2) ppm; ^1H NMR 7.89 (d, ortho), 7.50 (t, para), 7.40 (t, meta), 4.34 (s,CH_2) ppm; ^{11}B NMR 13.6 ppm relative to external $B(OMe)_3$. $PhB(O_2C_2H_4)F^-$, ^{13}C NMR 132.0 (ortho), 126.0 (meta), 124.5 (para), 63.6 (CH_2) ppm; ^1H NMR 7.43 (d, ortho), 6.83 (t, para), 6.94 (t, meta), 3.68 (s,CH_2) ppm; ^{11}B NMR -9.6 ppm relative to external $B(OMe)_3$.
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