Communications

Selective Transport of Riboflavin through a Liquid Organic Membrane

Edward Lambert, Eric C. Breinlinger, and Vincent M. Rotello*

University of Massachusetts, Amherst, Massachusetts 01003

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Riboflavin (vitamin B_2), a water soluble vitamin, is an essential factor in several metabolic pathways such as amino acid and lipid metabolism.[1] Additionally, it serves as a precursor for the enzymatic cofactors flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).[2] The sole source of riboflavin in mammals is dietary,[3] making efficient uptake and cellular transport of this molecule essential. Riboflavin deficiency has been shown to cause a large number of ailments including congenital malformations,[4] skin disorders,[5] and neuropathy,[6] as well as metabolic difficulties resulting from decreased activity of FMN- and FAD-dependent enzymes.[7] Ineffective cellular uptake of riboflavin has been implicated in a variety of ailments, including general malnutrition[8] and chronic renal failure.[9]

Riboflavin, due to the ribose chain, is very nonlipophilic (the partition coefficient between water and chloroform is 0.001), limiting simple diffusion through the cellular lipid bilayer.[10] Additional studies have shown that it is too large to diffuse through water-filled pores in the cell wall.[11] The identification of riboflavin carrier proteins[12] and previous inhibitor studies[13] indicates that cellular uptake occurs through receptor-mediated endocytosis. In an effort to solubilize and transport riboflavin into cells, complexes with a variety of inorganic and organic compounds have been formed with no apparent positive effect on absorption and cellular transport.[8,14]

To provide a possible system for trans-membrane transport of riboflavin, we have examined the use of arylboronic acids as carrier molecules. Previous research[15] has shown effective transport of glycosides and ribonucleosides through liquid membranes using these systems. We now report that phenylboronic acid (PBA) provides efficient and *selective* transport of riboflavin (relative to other sugars) across a liquid organic membrane.

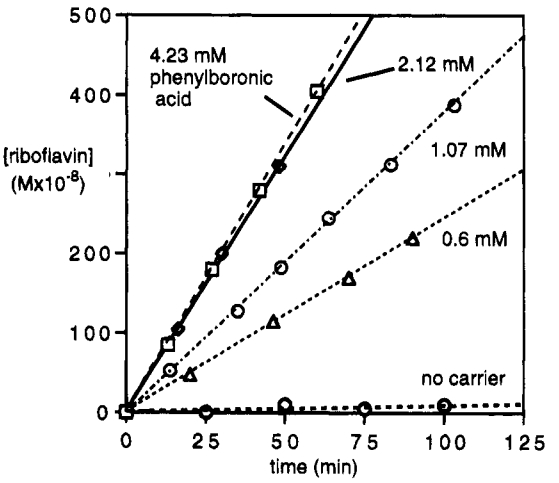

Figure 1. Change in riboflavin concentration in the receiving arm aqueous phase, using phenylboronic acid as carrier. All runs were in duplicate or triplicate, representative runs are shown here.

Riboflavin transport was quantified through standard downhill U-tube experiments.[15e,16] Absorbence measurements of receiving arm showed steadily increasing quantities of riboflavin transported and released (Figure 1).[17] Rate of transport varied with concentration of PBA, with maximal transport rates occurring with PBA concentration between 3 mM and 5 mM (Figure 2).[18] At these concentrations, the rate of riboflavin transport is enhanced by a factor of >200 over the unassisted process. Even at relatively low concentrations of PBA there is considerable enhancement in transport: 600 μM PBA provided a 70-fold enhancement of transport rate.

One important feature of the PBA-mediated transport of riboflavin is that no cationic cocarrier was required for efficient through-membrane transport.[19] This provides strong evidence that complexation between the ribose and PBA occurs through the neutral, trigonal,

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(16) A solution of phenylboronic acid in chloroform (7 mL) was placed in a U-tube apparatus. Then, 3.5 mL of 0.2 mM riboflavin in pH 7.4 phosphate buffer was added to one side of the tube, and 3.5 mL of pH 7.4 phosphate buffer was added to the other side. The organic layer was then stirred for 1 h (to equilibrate aqueous and organic phases), and then aliquots were removed from the receiving arm to determine riboflavin concentration.
(17) Absorbence measurements of the departure arm showed equal rates of riboflavin loss.
(18) At greater PBA concentrations riboflavin retention by the organic phase increased.
(19) Addition of cationic phase transfer agents such as TOMA (trioctylmethylammonium chloride) resulted in no measurable changes in riboflavin transport rates.

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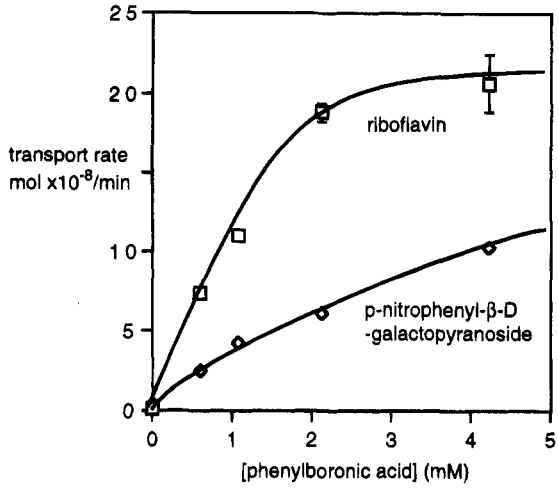
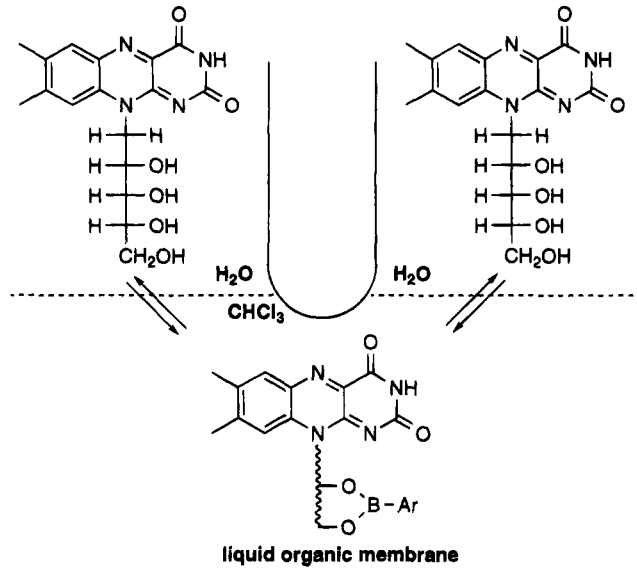


Figure 2. Rates of riboflavin and *p*-nitrophenyl β-D-galactopyranoside transport as a function of phenylboronic acid concentration. Rates shown are the average of two or three runs. Error bars represent the standard deviation between runs.

boronate ester (Scheme 1).[15e,20] In contrast, nucleosides and most other saccharides form tetrahedral, anionic boronate esters, which require a cationic cocarrier for efficient transport.[15,21] The neutral nature of the riboflavin boronate ester thus allows selective transport of riboflavin in the presence of molecules which form tetrahedral anionic complexes with phenylboronic acid.

Recent studies have shown that certain saccharides can be transported through their trigonal boronate esters.[15e] To examine the selectivity of PBA-promoted riboflavin transport relative to these saccharides, we examined the transport of *p*-nitrophenyl β-D-galactopyranoside (which was the most efficiently transported glycoside studied by Smith et al.).[15e] As shown in Figure 2, riboflavin transport occurred 3-fold faster than *p*-nitrophenyl galactoside[22] at PBA concentrations less than 3 mM. Since pnp-glycosides are considerably more lipophilic (and hence more easily transported) than their parent sugars, even greater selectivity of riboflavin transport by PBA would be expected in biological systems.

Scheme 1



In summary, we report the utility of phenylboronic acid in the selective complexation of riboflavin for the purposes of transport. PBA is an efficient carrier for riboflavin, increasing the rate of transport 200-fold over the unpromoted process. Additionally, transport of riboflavin by PBA was shown to be faster than other naturally occurring diols, providing the possibility of selective transport in medicinal applications. We are currently studying PBA transport of riboflavin in biological systems and will report these results in due course.

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Supplementary Material Available: Experimental procedures used for quantifying riboflavin transport (1 page).

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(20) The regiochemistry of boronate ester formation on the ribose chain is currently under investigation.

(21) An alternative strategy is to use a cationic arylboronic acid: ref 15c.

(22) *p*-Nitrophenyl galactoside transport experiments were performed under conditions identical to those of the riboflavin studies.