Pergamon

Tetrahedron Letters, Vol. 37, No. 35, pp. 6303-6306, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01385-8

Nucleotide Carrier Mixture with Transport Selectivity for Ribonucleoside-5'-phosphates

Jennifer A. Riggs,[a] Kathleen A. Hossler,[a] Bradley D. Smith,[a]* Michael J. Karpa,[b] Greg Griffin,[b] and Peter J. Duggan[b]*

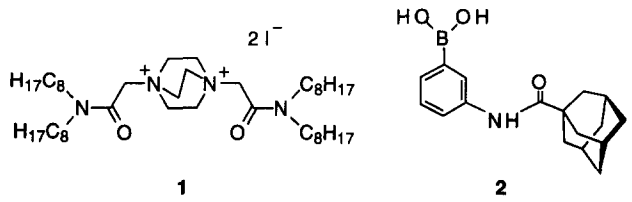
[a]Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556, USA.
[b]Department of Chemistry and Chemical Engineering, School of Molecular Sciences, James Cook University of North Queensland, Townsville, Queensland 4811, Australia.

Abstract: *A carrier mixture of lipophilic boronic acid and bis(quaternary ammonium cation) greatly facilitates the transport of ribonucleoside monophosphates through liquid organic membranes, at neutral pH. Transport selectivity was determined for the isomers of GMP. The observed order of non-competitive transport rates was 5'-GMP > 2'-GMP > 3'-GMP.* Copyright © 1996 Elsevier Science Ltd

The mono-, di-, and triphosphate derivatives of nucleosides are important biological compounds. They play major roles in processes ranging from signal transduction to gene replication. In addition, many nucleotide analogues possess important antiviral and anticancer activities. Under physiological conditions, nucleotides are charged entities and are unable to diffuse through cell membranes. Nature has solved this problem by using transport proteins that catalyze selective membrane transport (*e.g.*, ATP-ADP translocase in heart mitochondria).[1] It is thought that abiotic, but functionally mimetic, nucleotide transport carriers may be useful as drug delivery vehicles. As a consequence, a number of research groups are developing carriers that facilitate nucleotide transport through lipophilic membranes.[2-6]

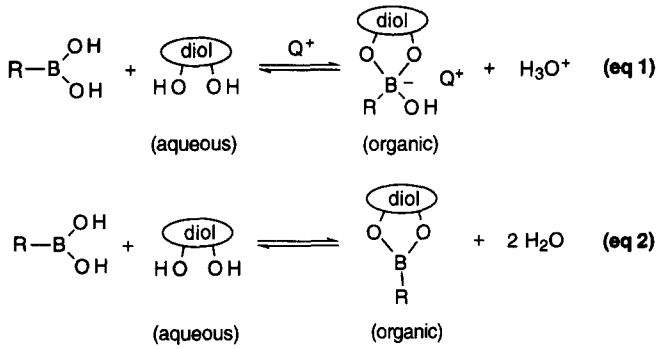
A ribonucleoside phosphate has three primary recognition sites; the phosphate, the nucleobase and the ribose diol. Nucleotide carriers have been reported with residues that have affinity for the anionic phosphate (ammonium, guanidinium, and expanded porphyrin cations) and the nucleobase (Watson-Crick, and Hoogsteen complements);[2-6] however, none have included a diol recognition motif. In a few cases, the isomeric selectivity of the carrier has been examined and a bias towards 2'- or 3'-phosphates over 5'-phosphates has been noted.[2,3] The most complete study to date has been conducted by Sessler who found that a sapphyrin-cytosine conjugate transported guanosine-2'-monophosphate (2'-GMP) ten times faster than its 5'- isomer.[2a] Here we describe a design strategy that reverses this isomeric selectivity. We have mixed a carrier that is selective for phosphate dianions with a carrier that is selective for *cis*-vicinal diols and produced a ribonucleotide transport system with 5'-monophosphate selectivity.

The phosphate carrier is compound **1**, a dicationic derivative of 1,4-diaza[2.2.2]bicyclooctane. This class of carrier compounds was originally introduced by Tabushi,[5] and later modified by Diederich,[4] who found that **1** facilitates the transport of nucleotides across a bulk, liquid membrane.[7] The diol carrier is boronic acid **2**, a lipophilic compound that condenses reversibly with diol-containing molecules (Scheme 1).[8] Initially, boronic acids were thought to mediate diol transport only by means of eq 1;[9] however, more recent studies have uncovered transport systems that use eq 2.[8,10,11]



1 2

Scheme 1



Liquid membrane transport was examined using a standard U-tube apparatus that has been described previously.[8] In this configuration an aqueous source phase is separated from an aqueous receiving phase by a layer of chloroform.[12] Appearance of the nucleotide in the receiving phase was monitored by UV absorption, and verified in certain cases by HPLC. The measured nucleotide fluxes in the presence and absence of carriers **1** and **2** are given in Table 1.

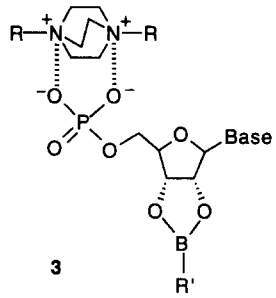
Table 1. Nucleotide Transport Through BLMs Containing Different Carriers.[a]

		initial flux[b] (rel flux)[c]			
entry	solute	no carrier	**1**	**2**	**1 + 2**
1	5'-ATP	0.02 (1)	92 (4600)	0.16(7)	68 (3400)
2	5'-ADP	0.08 (1)	48 (600)	0.52 (7)	156 (1950)
3	5'-AMP	0.18 (1)	3.1 (17)	0.64 (3.6)	35 (193)
4	5'-GMP	0.08 (1)	0.68 (8)	0.24 (3)	21 (248)
5	5'-UMP	0.20 (1)	1.6 (8)	0.64 (3)	27 (131)
6	2'-deoxy-5'-AMP	6.5 (1)	20 (3)	7.8 (1.2)	18 (2.8)
7	2'-deoxy-5'-GMP	0.11 (1)	1.0 (9)	0.71 (6)	1.1 (10)
8	2'-deoxy-5'-UMP	0.10 (1)	3.0 (30)	1.4 (14)	2.1 (21)

[a]Source phase: potassium phosphate (100 mM, pH 7.3), nucleotide (10 mM); Liquid membrane: carrier(s) (1 mM) dissolved in chloroform; Receiving phase: potassium phosphate (100 mM, pH 7.3); Temperature: 295 °K. [b]Average of at least two runs; 10^{-9} mol/m^{2}s ± 15 %. [c]Relative to the background flux observed in the absence of carrier.

In agreement with Tabushi,[5] and Diederich,[4] carrier **1** facilitated adenosine phosphate transport in the following order: 5'-ATP (4600 times faster than background diffusion, entry 1) > 5'-ADP (600 times faster than background diffusion, entry 2) >> 5'-AMP (17 times faster than background diffusion, entry 3). Low transport enhancements were also observed with other nucleoside monophosphates (entries 4 - 8).

On its own, the lipophilic boronic acid **2** had a weak or negligible effect on transport rates. The carrier mixture of **1+2**, on the other hand, produced a synergistic effect, particularly in the case of ribonucleoside-5'-monophosphate transport. Compared to the fluxes induced by carrier **1**, the **1+2** mixture increased transport by factors of eleven (5'-AMP, entry 3), thirty-one (5'-GMP, entry 4), and seventeen (5'-UMP, entry 5). With 2'-deoxyribonucleoside-5'-monophosphates, the **1+2** carrier mixture was no more effective than carrier **1** alone (entries 6 - 8). This suggests that in the case of ribonucleoside-5'-monophosphates the transported species is the ternary complex **3**.[9b,11] The strong transport selectivity in favor of ribonucleoside-5'-monophosphate over 2'-deoxyribonucleoside-5'-monophosphate contrasts with the selectivity of Sessler's expanded porphyrins which are relatively insensitive to diol configuration (the transport rates for ribo-, 2'-deoxyribo-, and arabinonucleoside-5'-monophosphates differed by less than 50 %).[2c,2d]



3

Although the **1+2** mixture transported 5'-AMP eleven times better than carrier **1**, it was only three-fold more effective with 5'-ADP (entry 2), and actually inhibited 5'-ATP transport (entry 1). These results are attributed to a change in the rate-determining step for the different processes. In the case of 5'-AMP transport, the rate-determining step is entry of the nucleotide into the organic membrane and formation of a moderately organic-soluble complex with 5'-AMP:**1** stoichiometry of 1:1. With 5'-ATP, however, the rate-determining step is nucleotide release from the membrane because the transported species is a highly organic-soluble complex with 5'-ATP:**1** stoichiometry of 1:2.[4] Support for this rationalization was provided by transport experiments which examined the change in nucleotide levels in both the source and receiving phases. These experiments showed that the initial loss of 5'-ATP from the source phase using either carrier **1** or a **1+2** mixture is about twice as fast as the rate of steady-state release into the receiving phase. In other words, both carrier systems suffer from substantial build up of carrier-substrate complex in the organic membrane (*i.e.*, $K_{ex} > K_{ex(max)}$).[10] Under these conditions, improving 5'-ATP extraction ability by changing from **1** to a **1+2** mixture does not produce an increase in observed transport flux.

The assignment of the transported species as **3** is based in part on the known preference of boronic acids for *cis*-vicinal diols.[10] Since boronic acid complexation with other diol configurations is less favored, the **1+2** carrier mixture was predicted to be less effective with 2'- and 3'-substituted ribonucleoside monophosphates. This was tested in the case of the isomers of guanosine monophosphate. The observed order of non-competitive

transport rates was 5'-GMP (2.1×10^{-8} mol/m^{2}s) > 2'-GMP (5.1×10^{-9} mol/m^{2}s) > 3'-GMP (2.9×10^{-9} mol/m^{2}s). This selectivity order is the reverse of the Sessler,[2] and Rebek[3] carriers, and thus expands the current "portfolio" of abiotic nucleotide transport systems.

In conclusion, a carrier mixture of lipophilic boronic acid and bis(quaternary ammonium cation) greatly facilitates the selective membrane transport of ribonucleoside-5'-monophosphates at neutral pH. An obvious progression in the design is to covalently link the two carrier components together. A further extension is to combine the known recognition motifs for phosphate, nucleobase, and vicinal diol to produce a receptor with very high 5'-ribonucleotide selectivity.

Acknowledgments. This work was supported by the US National Science Foundation, the Australian Department of Industry, Science and Technology and the Australian Research Council. M. J. K. is a recipient of an Australian Postgraduate Research Award.

References and Notes

1. Powell, S. J.; Medd, S. M.; Runswick, M. J.; Walker, J. E. *Biochemistry* **1989**, *28*, 866-873.
2. (a) Král, V.; Sessler, J. L. *Tetrahedron* **1995**, *51*, 539-554. (b) Král, V.; Andrievsky, A.; Sessler, J. L. *Chem. Commun.* **1995**, 2349-2351. (c) Sessler, J. L.; Furuta, H.; Král, V. *Supramol. Chem.* **1993**, *1*, 209-220, and references cited therein. (d) Furuta, H.; Cyr, M. J.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 6677-6678.
3. Andreu, C.; Galán, A.; Kobiro, K.; de Mendoza, J.; Park, T. K.; Rebek, J.; Salmerón, A.; Usman, N. *J. Am. Chem. Soc.* **1994**, *116*, 5501-5502.
4. (a) Li, T.; Krasne, S. J.; Persson, B.; Kaback, H. R.; Diederich, F. *J. Org. Chem.* **1993**, *58*, 380-384. (b) Li, T.; Diederich, F. *J. Org. Chem.* **1992**, *57*, 3449-3454.
5. Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am.Chem. Soc.* **1981**, *103*, 6152-6157.
6. Yasuhisa, K.; Hatekayama, H.; Seshimo, H.; Ogoshi, H. *Supramol. Chem.* **1994**, *3*, 267-271.
7. Diederich actually used the dibromide salt of **1**, however, we found that its extremely hydrogscopic nature made handling difficult.[4] As the bis(iodide) salt, **1** is much easier to work with. It absorbs at 218 nm which complicates organic extraction experiments, but we encountered no problems using it in membrane transport studies.
8. Morin, G. T.; Paugam, M. -F.; Hughes, M. P.; Smith, B. D. *J. Org. Chem.* **1994**, *59*, 2724-2728.
9. (a) Shinbo,T.; Nishimura, K.; Yamaguchi, T.; Sugiura, M. *Chem. Commun.* **1986**, 349. (b) Grotjohn, B. F.; Czarnik, A. W. *Tetrahedron Lett.* **1989**, *30*, 2325-2328. (c) Mohler, L. K.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 2998-2999.
10. (a) Smith, B. D. *Supramol. Chem.* **1996**, *7*, 55-60 and references cited therein. (b) Smith B. D. In *Chemical Separations with Liquid Membranes*, Bartsch, R. A., Way, J. D., Eds.; ACS Symposium Series, ACS: Washington, 1996.
11. Westmark, P. R.; Smith, B. D. *J. Pharm. Sci.* **1996**, *75*, 266-269.
12. The aqueous source and receiving phases (3.5 mL, 100 mM potassium phosphate, pH 7.3) were carefully placed on top of a solution of carriers(s) in chloroform (1 mM, 7 mL) located at the bottom of a U tube apparatus (1.2 cm internal diameter, 10 cm high, 2.5 cm between each arm) which was equipped with a stir bar and a magnetic stirrer (stirring rate 475 rpm).

(*Received in USA* 11 *June* 1996; *accepted* 11 *July* 1996)