Transport of Adenine Mono- and Dinucleoside Monophosphates across Liquid Membranes and Extraction of Oligonucleotides with Synthetic Carriers

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Received December 13, 1993

Carriers for transport of nucleotides across model membranes have been developed from lipophilic quaternary ammonium salts[2,3] or from principles of molecular recognition.[4] We recently introduced synthetic receptors for adenylic acids that involve molecular recognition through hydrogen bonding, aromatic stacking effects, and salt bridges.[5] These molecules have now been further refined to yield highly lipophilic molecules that show good activity for selective transport of short nucleotides across liquid membranes and extraction of longer oligonucleotides into organic solvents.

The molecules **1a**, **1b**, and **2** were assembled from Kemp triacid derivatives,[6] carbazole spacer subunits,[5,7] and guanidinium complements for phosphates,[8,9] according to described synthetic routes.[5,10] Optically pure guanidinium derivatives, having the *S,S* configuration, were used in all cases. The transport studies used a simple U-tube apparatus in which 1,2-dichloroethane (DCE) represented a liquid membrane between two aqueous phases.[11] The source phase contained the nucleotides at the concentrations indicated in Table 1; transport (and *active* transport) was promoted by 10 mM sodium chloride in the receiving phase.

Carrier **2** showed the highest selectivity for mononucleotides of adenine *vs* guanine, but it is likely that the lower solubility of guanine mononucleotide in the organic phase contributes to some of the observed selectivity. In addition, some preference for 2′,3′-cyclic AMP transport *vs* 3′,5′-cyclic AMP was apparent. Even 3′-AMP could be transported—presumably as the monoanion[12]—with increased concentrations of carrier. Control experiments with **3**, a lipophilic guanidinium lacking the adenine binding modules, support the premise that molecular recognition is responsible for the selectivity of these carriers.[13]

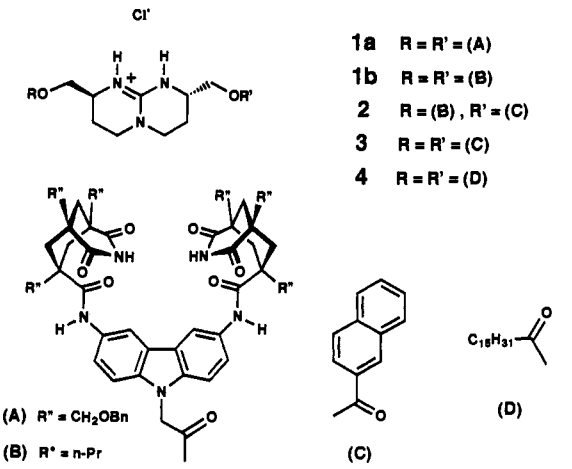
For the dinucleoside monophosphate carriers **1a** and **1b**, the most effective guest was d(ApA). Other adenine-containing dinucleoside monophosphates could also be transported, d(ApT), d(TpA), and d(ApG), but the mere presence of adenine was not sufficient, as neither d(ApC) nor d(CpA) was transported. Again, experiments with controls **3** and **4** showed no transport.

Table 1. Transport of Nucleotides by Receptors[a]

nucleotide	carrier	concn (μM)	initial transport[b] rate (10^{-9} mol cm^{-2} h^{-1})	active transport[b] rate (10^{-9} mol cm^{-2} h^{-1})
2′,3′-cAMP	**2**	7.5	2.194 ± 0.016	1.216 ± 0.004
2′,3′-cAMP	blank		none (in 9 h)	
2′,3′-cAMP	**3**	7.5	none (in 9 h)	
2′,3′-cAMP	**3**	20	none (in 9 h)	
3′,5′-cAMP	**2**	7.5	1.820 ± 0.040	0.964 ± 0.013
2′,3′-cGMP	**2**	7.5	none (in 40 h)	
3′,5′-cGMP	**2**	7.5	none (in 20 h)	
3′-AMP	**2**	25	1.383 ± 0.037	0.685 ± 0.023
3′-AMP	**3**	20	none (in 13 h)	
5′-AMP	**2**	25	none (in 9 h)	
d(ApC)	**1a**	10	none (in 24 h)	
d(CpA)	**1a**	10	none (in 10 h)	
d(CpG)	**1a**	10	none (in 24 h)	
d(GpG)	**1a**	20	none (in 9 h)	
d(ApA)	**1a**	5	1.027 ± 0.0041	0.755 ± 0.018
d(ApA)	**1b**	5	1.221 ± 0.019	0.787 ± 0.035
d(ApA)	blank		none (in 9 h)	
d(ApA)	**3**	20	none (in 24 h)	
d(ApA)	**4**	20	none (in 9 h)	
d(ApT)	**1a**	12	1.058 ± 0.053	0.792 ± 0.041
d(TpA)	**1a**	12	0.910 ± 0.01	0.484 ± 0.024
d(ApG)	**1a**	20	0.360 ± 0.004	not determined

[a] No leakage of receptors (25 μM) was observed in blank experiments. The concentration of nucleotide in the source phase (2.0 mL) was 15 μM for mononucleotide transport and 10 μM for dinucleoside monophosphate transport. The receiving phase was 2.0 mL of a 10 mM solution of sodium chloride. The bulk membrane consisted of 6.0 mL of DCE. For a description of the transport cell, see note 11. [b] All experiments were performed at least twice. Upper and lower limits are represented as (±) deviations from the mean values.

Chart 1



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(10) All new compounds (**1a**, **4**) were characterized by a full complement of high-resolution NMR spectra. For compounds **1b**, **2**, **3**, and **4**, see refs 5a, 5b, 9a, and 9b, respectively.

(11) A glass U-tube of 4.0-cm radius and two 8.0-cm branches (outer dimensions), with a contact surface (inner area) of 1.568 cm^2, was used as transport system. The system was stirred with a magnetic bar (1.0 × 0.3 cm) at 1200 rpm. The transport was monitored measuring periodically the absorbances at 260 nm in both the source and receiving phases. The experimental values of the receiving phase showing straight lines were adjusted to linear equations to calculate the rate constants (initial and active transport).

(12) At the source initial pH (5.6), most of the 3′-AMP (pK_a 6.7) is monoanionic. A control experiment run at pH 8.0 (dianion) required 20 h to get the same amount transported at pH 5.6 in only 3 h.

0002-7863/94/1516-5501$04.50/0

Table 2. Extraction of Nucleic Acids by Receptor **1a**[a]

sequence[b]	length	MW	NA concn μM	receptor concn μM	receptor/NA	% extracted	% released
AA	2	660	50	50	1/1	35	43
d(AAA)	3	990	50	50	1/1	29	60
d(AAA AA)	5	1650	25	25	1/1	21	34
d(TAT ATA)	6	1980	12	12	1/1	11	26
d(AAA AAA A)	7	2310	12	12	1/1	14	16
d(TTT TTA A)	7	2310	12	12	1/1	10	30
d(GCA TTA A)	7	2310	12	12	1/1	6	40
d(TTT GGA A)	7	2310	12	12	1/1	13	23
$d(A_8)$	8	2640	12	12	1/1	12	30
$d(T_8)$	8	2640	12	12	1/1	<5	
CCC UCU AAA AA	11	3630	10	12	1.2/1	37	28
$(AG)_8$-dT	17	5610	6	12	2/1	23	8
HH ribozyme ...d(GC)	19	6270	6	12	2/1	17	12
HH riboyme ...GC	19	6270	6	12	2/1	27	10
A_{20}	20	6600	6	12	2/1	68	10
RNA PCR primer ...GG	20	6600	6	12	2/1	20	17
HH substrate ...GC	24	7920	6	25	4/1	24	4
RNA PCR primer ...CC	24	7920	6	25	4/1	24	
HH ribozyme ...GU	35	11 550	6	25	4/1	10	2
HH ribozyme ...d(GT)	35	11 550	6	25	4/1	13	6
sun Y ribozyme ...UC	35	11 880	6	25	4/1	12	6
tRNA alanine ...CCA	76	25 080	1.5	12	8/1	6	6

[a] For details on the extraction, see note 14. NA = nucleic acid; oligoribonucleotides and RNA/DNA chimeras were synthesized according to ref 15. [b] HH ribozyme ...d(GC): 5′ GGG UCG ACU GAU GAG GC(dG dC) 3′; HH ribozyme ...GC: 5′ GGG UCG ACU GAU GAG GCG (dC) 3′; RNA PCR primer ...GG: 5′ AGG CAU ACU AGU ACA AGU GG 3′; HH substrate ...GC: 5′ GCG CCG AAA CAC CGU GUC UCG AGC 3′; RNA PCR primer ...CC: GGA ACU UAG CGU GAA UUC GAU CCC 3′; HH ribozyme ...GU: 5′ GCU CGU CUG AUG AGU CCG UGA GGA CGA AA(dG) ACC GU 3′; HH ribozyme ...d(GT): 5′ d(GCT CGT CT(rG) AT(rG) (rA)GT CCG TGA GGA CGA AA(rG) ACC GT) 3′; sun Y ribozyme ...UC: 5′ GCU GUA AAU GCC UAA CGA CUA CAC GGU AGA CAA CUC 3′.

In two-phase single extraction experiments, the highly lipophilic compound **1a** was remarkably effective in removing *long* oligonucleotides from dilute aqueous solutions into DCE (Table 2). A systematic study on selectivities has yet to be done, but it appears that the following are requirements for successful extraction: some adenosines must be present in the sequence, and the number of adenosines in general and ApA repeats in particular correlate with increased extraction efficiency. This was illustrated in the case of A_{20} (20-mer), in which 68% was extracted, while both the HH ribozyme ...GC (19-mer) and RNA PCR primer ...GG (20-mer) (sequences of comparable length but much lower adenosine and ApA content) were extracted at 27% and 20% efficiencies, respectively (at the same 2:1/receptor:NA ratio). Even tRNA was extracted to a detectable extent when exposed to excess carrier in the organic phase. The complexes that are formed can be partly dissociated by a single backwash. The percent released in Table 2 refers to the calculated amount of nucleotide in the organic phase that reappears in the aqueous wash phase.

In conclusion, we have shown that nucleoside monophosphate transport can be obtained in model liquid membranes with synthetic receptors. These results augur well for developing carriers appropriate for the transport of therapeutic agents such as phosphorylated versions of nucleotide analogs (AZT and DDI) across biological membranes. They also suggest that synthetic carriers may be developed to deliver antisense and ribozyme oligonucleotides.

Acknowledgment. We thank the National Institutes of Health and the National Aeronautics & Space Administration (N.U.) for financial support and Professor Alexander Rich for his encouragement and the hospitality of his laboratories.

(13) Binding enthalpies were examined with molecular mechanics and molecular dynamics calculations on the d(AA)-**1b** complex. These indicated that *ca.* 25% of the binding energy results from the guanidinium–phosphate salt bridge, *ca.* 30% from the Kemp triacid modules, and *ca.* 30% from carbazole–adenine stacking interactions: de Mendoza, J.; Gago, F. In *Computational Approaches in Supramolecular Chemistry*; Wipff, G., Ed.; Kluwer; Dordrecht, in press.

(14) Extraction procedure: the nucleotides were dissolved at the concentrations indicated in 200 μL of H_2O or NH_4OAc buffer, 50 mM (pH 7), and the absorbance at 260 nm was measured. The solution was removed from the UV cuvette and added to 200 μL of a 50 μM solution of **1a** in DCE. The mixture was vortexed for 30 s in a 500-μL Eppendorf tube, and the two phases were separated by centrifugation at 10 000 rpm for 30 s. The DCE layer was removed, and the absorbance of the remaining aqueous layer was measured. $(A_{260}$ after $- A_{260}$ before$)/A_{260}$ before = % extracted. To determine the amount released, the DCE solution containing the extracted oligonucleotide was extracted with 200 μL of H_2O or buffer, and the absorbance of the aqueous extract was measured at 260 nm. A_{260} after$/(A_{260}$ before $- A_{260}$ after) = % released.

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