Phosphate Anion Binding: Enhanced Transport of Nucleotide Monophosphates Using a Sapphyrin Carrier

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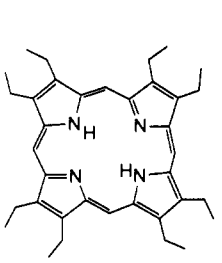
Received June 4, 1991

Recently, nucleotide analogues have been the focus of considerable attention because of their potential utility in antiviral chemotherapy. For example, the monophosphate derivatives of both 9-(β-D-arabinofuranosyl)adenine (Ara-AMP) and 9-(β-D-xylofuranosyl)guanine (Xylo-GMP) have been shown to have high anti-HSV activity in cell-free suspensions,[1] and phosphate analogues such as phosphonate and/or phosphonyl methoxy ether nucleobase derivatives are known to have antiviral activity in vitro.[2] However, because of their charged nature, such nucleotide analogues generally have poor penetration into cells.[3] Carriers which could enhance cellular uptake of nucleotide agents might, therefore, have an important role to play as adjuvants to antiviral chemotherapy. In addition, they might also be of interest in terms of understanding, from a model point of view, the bioenergetics of more normal through-membrane nucleotide transport.[4]

In early work, Tabushi was able to effect adenosine nucleotide transport with a lipophilic quarternary amine system.[5] However this same system failed to mediate the transport of guanosine 5′-monophosphate (GMP) or other guanosine-derived nucleotides. In fact, we are currently unaware of any system capable of effecting the through-membrane transport of these highly hydrophilic species. Recently, we have reported the selective transport of *nucleosides* with synthetic lipophilic carriers[6] as well as the synthesis of several rationally designed GMP receptors which proved capable of effecting recognition in DMSO but not through-transport in a U-tube type, H_2O–$CHCl_3$–H_2O, model membrane system.[7] We now wish to report the successful transport of GMP and other nucleotide monophosphates with sapphyrin **2** as the carrier.

Sapphyrin (Sap, **2**) is a 22 π-electron pentapyrrolic "expanded porphyrin" which can exist as H_3Sap, H_4Sap^+, and H_5Sap^{2+} according to its degree of protonation.[8] From a single-crystal X-ray analysis and several followup solution studies,[9] it was determined that H_5Sap^{2+} can bind fluoride anion (F^-) in the solid state and this and several other anions in solution. More recently, on the basis of CPK model studies, we have considered that



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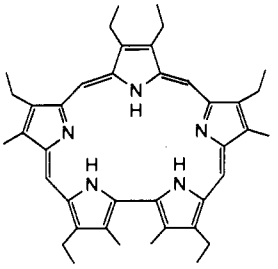

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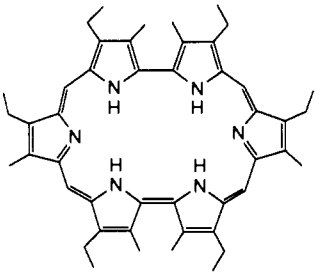

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phosphate anion may be capable of binding to H_5Sap^{2+} via well-oriented pyrrole NH to phosphate oxygen hydrogen bonds. As phosphate binding is clearly an important aspect in the design of a solubilizing nucleotide carrier, we considered that H_5Sap^{2+} might act as an effective agent for the through-membrane transport of GMP and related systems. To test this hypothesis, we have carried out GMP transport studies with a simple three-phase (H_2O–CH_2Cl_2–H_2O) model membrane system.

Prior to the transport experiments, the pK_a values of the sapphyrin **2** were determined and found to be $pK_{a1} \cong 3.5$ (H_5Sap^{2+}/H_4Sap^+) and $pK_{a2} \cong 9.5$ (H_4Sap^+/H_3Sap).[10] Thus, GMP transport was expected to be most efficient at <pH 3.5 since, under these conditions, good interactions between H_5Sap^{2+} and GMP^{2-} were expected to pertain. Initial transport experiments, therefore, were carried out at pH 2.5 with GMP diacid in the initial aqueous phase (Aq I) and the free-base form of sapphyrin in the CH_2Cl_2 phase. In the absence of carrier, GMP was not detected in Aq II (initial pH = 10.0) even after 3 days. On the other hand, a steady buildup of GMP in Aq II was observed, without any significant induction period, when the sapphyrin was present in the organic phase (Table I). As the pH of Aq I was raised, the rate of GMP transport dropped, becoming insignificant at pH ≥ 4.0 (Table I). With the same experimental setup, a number of other agents, including octaethylporphyrin (OEP, **1**), distearyl diazabicyclooctane dichloride (C_{18}-dabco),[5] and triisopropylsilyl-protected cytidine (C-Tips)[6] were also tested as possible carriers. None, however, proved effective.

During the above sapphyrin-mediated GMP transport, the pH of Aq II was found to decrease. This suggests a so-called symport[11] process in which both H^+ and GMP^{2-} are bound to the carrier (H_3Sap), carried through the organic liquid membrane in concert, and then released into the basic environment of Aq II (Scheme I). Left undefined, however, by these observations is the exact nature of the transported species. On the basis of the observed pH rate dependence (Table I) and independent ^1H NMR experiments,[12] we are tempted to assign this species to a tightly ion

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(10) pK_a values were determined by monitoring the absorbance change at 451.5 nm occurring upon mixing a CH_2Cl_2 solution of $[H_5Sap^{2+}\cdot 2Cl^-]$ (1×10^{-6} M) and a pH-adjusted NaCl aqueous solution ($[Cl^-]_{total} = 50$ mM; HCl for pH < 7, NaOH for pH > 7); cf. supplementary material.

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(12) When GMP diacid powder was added to an H_2O-saturated $CDCl_3$ solution of H_3Sap, the broad singlet signal (at 11.5 ppm) of the meso-like sapphyrin methine protons was replaced by two sharp peaks at 11.66 and 11.70 ppm, which are typical signals of H_5Sap^{2+} complexes.

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Table I. Initial Transport Rate[a]

substrate	carrier	pH (Aq I)[b]	initial transport rate 10^{-9} mol/(cm²·h)
GMP	OEP (1)	2.5	≤0.001
	sapphyrin (2)	2.5	86.9
		3.0	51.9
		3.5	22.9
		4.0	1.87
	rubyrin (3)	2.5	16.6
AMP	sapphyrin	3.1	262.1
Ara-AMP	sapphyrin	3.1	183.9

[a] Transport experiments were performed in a manner similar to those reported in ref 6: [substrate] = 10 mM and [carrier] = 0.1 mM. Initial transport rates were calculated from the linear region of concentration vs time curve (cf. supplementary material). Estimated errors ≤5%. [b] The pH of Aq I was adjusted by adding 1 N NaOH to the GMP diacid solution. Aq II was basified with NaOH to give an initial pH = 10.0.

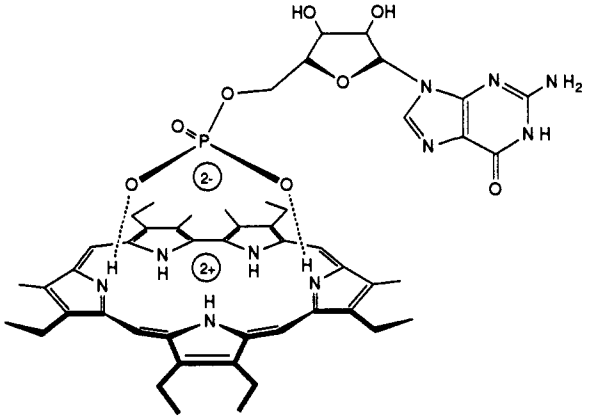
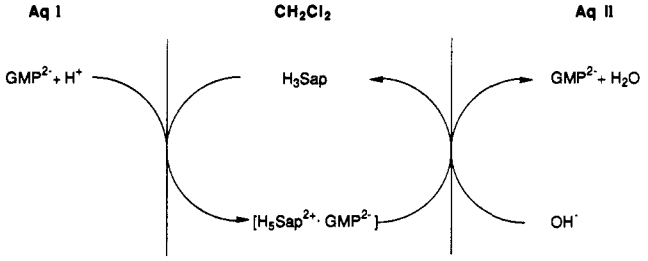


Figure 1. Schematic representation of proposed 1:1 complex formed between sapphyrin 2 and GMP diacid. Under the conditions of transport, higher order aggregates and sapphyrin species with different degrees of protonation could be contributing; see text.

Scheme I



paired, *overall neutral* [H_5Sap^{2+}·GMP^{2-}] complex (Figure 1). However, the participation of other entities, including dimers such as [H_4Sap^+·GMP^{2-}·H_4Sap^+] and/or other higher aggregates[13] cannot at present be entirely ruled out.[14]

Sapphyrin **2** also appears to effect transport of other monophosphate species. Both AMP and Ara-AMP, for instance, are transported efficiently. In fact, as might be expected for these more lipophilic adenosine derivatives, the actual transport rates are slightly higher than those observed for GMP (Table I). Interestingly, the transport of both AMP and GMP appears to be inhibited by certain other small anions. The addition, for instance, of NaCl or NaF (1-2 molar equiv relative to Sap) to Aq I leads to long induction periods (1-2 h) for nucleotide transport. Presumably, this reflects the fact that these anions are also bound and transported by the protonated forms of sapphyrin.[15]

In conclusion, we have demonstrated effective transport of nucleotides and analogues through a dichloromethane membrane with protonated sapphyrin as the carrier. Currently, we are exploring the range and scope of this expanded porphyrin approach to nucleotide transport. In preliminary work, we have found that the new rubyrin system (**3**)[16] is able to transport GMP but is less effective than sapphyrin **2** for this purpose (Table I). However, this larger macrocyclic system appears relatively more effective for the transport of diphosphorylated species such as, e.g., GDP.[17] This leads us to suggest that specific structural effects could be important in regulating this general expanded porphyrin approach to nucleotide transport and recognition.

Acknowledgment. This work was supported by the Texas Advanced Research Program (Grant No. 3658-016). J.L.S. also thanks the National Science Foundation (PYI 1986), the Camille and Henry Dreyfus Foundation (Teacher-Scholar Award 1988-1993), and the Sloan Foundation (Fellowship 1989-1991).

Supplementary Material Available: pH titration curve for sapphyrin and time course of GMP, AMP, and Ara-AMP transport (3 pages). Ordering information is given on any current masthead page.

(13) Since the free-base sapphyrin (H_3Sap) is strongly basic, it generates the mono cation [H_4Sap^+·OH^-] in the presence of a small amount of water. In the presence of larger amounts of water, dimerization and/or aggregation occurs to give a species with λ_{max} = 461.5 nm. Similar effects have been observed in the diacid (H_5Sap^{2+}) form: Maiya, B. G.; Cyr, M. J.; Harriman, A.; Sessler, J. L. *J. Phys. Chem.* **1990**, *94*, 3597-3601.

(14) During transport, the absorption maximum of the CH_2Cl_2 layer in the U-tube was found to shift from 446 to 450 nm; this is consistent with the existence of both mono- and diprotonated sapphyrin species.

(15) Fluoride anion transport is also enhanced by sapphyrin: Sessler, J. L.; Ford, D.; Cyr, M. J.; Furuta, H. To be submitted for publication.

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Palladium-Mediated Stereocontrolled Reductive Amination of Azido Sugars Prepared from Enzymatic Aldol Condensation: A General Approach to the Synthesis of Deoxy Aza Sugars[1]

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Received March 5, 1991

We report in this study the diastereoselective Pd-mediated reductive amination of azido ketoses or aldoses prepared (on 1-10-mmol scales) from aldolase reactions to five- or six-membered-ring deoxy aza sugars. Although only four aldolases have been used in this study, it appears to be general that this combined chemical and enzymatic approach is a very effective way for the construction of deoxy aza sugars structurally related to many natural and unnatural monosaccharides.

Aza sugars[2] are useful inhibitors of enzymes associated with carbohydrate processing.[2,3] Synthesis of aza sugars based on

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