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Complexation of Nucleotides in Water with Cyclotetrachromotropylene

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Abstract. The stability constants K of the 1 : 1 host to guest complexes formed between cyclotetrachromotropylene and the two nucleotides, uridine 2′,3′-cyclicmonophosphate, sodium salt and adenosine 2′,3′-cyclicmonophosphate, sodium salt, in water were determined by proton NMR spectroscopy. They are 35 and 195 M^{-1}, respectively, at 25 °C. The base component of each nucleotide is included in the hydrophobic cavity of the host in the two complexes.

Key words: cyclotetrachromotropylene, nucleotides, complexation, stability constant, macrocycle, molecular recognition

1. Introduction

The study of molecular recognition in an aqueous medium is growing in importance because it provides an understanding of the chemistry in a biological system. Various macrocycles have been used to complex with a variety of guest molecules and ions [1–3]. However, relatively few studies with nucleotides as guests have been reported [4–7].

There are two ways for a macrocycle to complex with a nucleotide. The first one is through electostatic attractions between the cationic sites in the macrocycle and the anionic phosphate groups of the sugar moiety [5]. The second way is by including the more hydrophobic base moiety of the nucleotide in the hydrophobic cavity of the host [7]. Since the water-soluble cyclotetrachromotropylene (**1**) has a large hydrophobic cavity [8] we were interested in studying its complexation with nucleotides. We chose two nuleotides, uridine 2′,3′-cyclicmonophosphate, sodium salt (**2**) and adenosine 2′,3′-cyclicmonophosphate, sodium salt (**3**) for our study. The former as a representative of a nucleotide with a pyrimidine base and the latter a purine base. This paper reports the results of our proton NMR study on the complexation of these two nuleotides with **1** in water at 25 °C.

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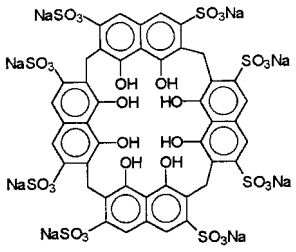


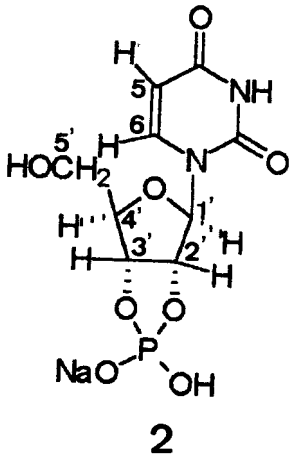
1

Scheme 1.

2. Experimental

2.1. MATERIALS

Compound **1** was synthesized according to the method reported earlier [9]. Nucleotides **2** and **3** were commercial samples.



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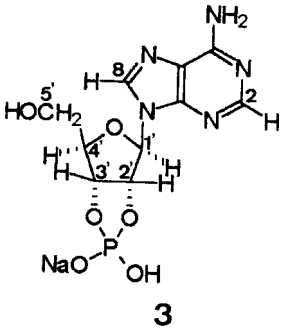

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Schemes 2–3.

2.2. PROTON NMR SPECTRA

Proton NMR spectra in D_2O at 25 °C were recorded with a 300 MHz Bruker AC300 Superconducting NMR spectrometer. The solvent peak (unaffected by the concentration variation of the host and guest compounds) at 4.80 ppm was used as the internal reference. In all the chemical shift titrations, the concentration of the nucleotides was kept constant (2.895×10^{-2} M and 1.730×10^{-2} M for **2** and **3** respectively) while the concentration of the host **1** varied.

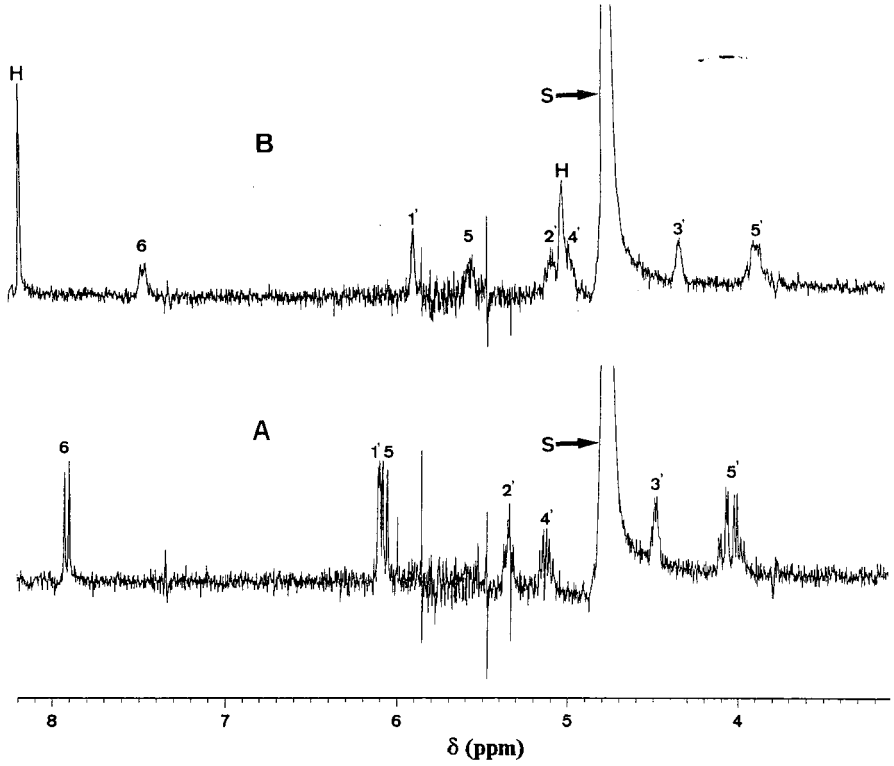


Figure 1. 300 MHz proton NMR spectra in D_2O at 25 °C of 2.895×10^{-2} M of nucleotide **2** (solvent peak **S** at 4.80 ppm as internal reference); (**A**) no host, (**B**) in the presence of 2.246×10^{-2} M of host **1** (host resonance peaks indicated by **H**).

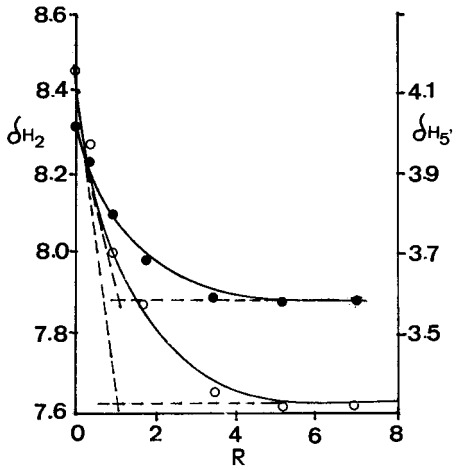


Figure 2. Calculated proton chemical shift titration curves of H_2 and $H_{5'}$ of 1.730×10^{-2} M of nucleotide **3** in D_2O at 25 °C. The K and δ values of the free and complexed guest used for calculating the titration curves are given in Table I. *R* is the molar ratio of host to guest used and the points are experimental values.

Table I. Proton chemical shifts of guests **2** and **3** and their 1 : 1 host to guest stability constant K in water at 25 °

Proton	δ_u (ppm)[a]	$\Delta\delta$ (ppm)[b]	$\Delta\delta_{max}$ (ppm)[c]	K (M^{-1})
Guest **2**				
H_5	6.07	0.50	nd	
H_6	7.92	0.46	1.42	25
$H_{1'}$	6.11	0.20	0.55	45
$H_{2'}$	5.34	0.25	0.54	
$H_{3'}$	4.49	0.14	0.32	
$H_{4'}$	5.12	0.12	nd	
$H_{5'}$	4.00	0.12	0.30	35
Guest **3**				
H_2	8.46	0.53	0.85	190
H_8	8.42	0.48	0.80	
$H_{1'}$	6.37	0.26	0.56	
$H_{2'}$	5.55	0.34	0.50	
$H_{3'}$	4.60	0.21	nd	
$H_{4'}$	5.28	nd	nd	
$H_{5'}$	4.01	0.22	0.37	200

[a]Chemical shift of free guest.
[b]Induced chemical shift at molar ratio of host to guest of 0.8.
[c]Maximum induced chemical shift.
nd = resonance peak not discernable.

2.3. STABILITY CONSTANT

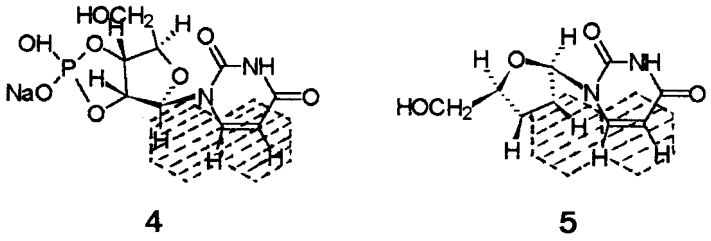
Calculations of the stability constant K of the 1 : 1 host to guest complexes using the nonlinear regression fitting of the proton chemical shift titration curves were carried out as reported earlier [9].

3. Results and Discussion

The two nucleotides complexed with the host **1**, as indicated by the upfield shift of their proton NMR chemical shifts upon addition of **1** (Figure 1 shows the case for nucleotide **2**). The NMR chemical shift titration plots (Figure 2 for nucleotide **3**) indicate that the complex is of 1 : 1 host to guest stoichiometry since the two tangents to each of the titration curves meet at a point where the molar ratio *R* is one [9]. The larger induced chemical shifts for the protons of the pyrimidine (H_5 and H_6 of **2**) and purine rings (H_2 and H_8 of **3**), compared to those for the protons of the sugar unit (Table I), may indicate that the base moiety is included in the hydrophobic cavity of the host in each of the complexes. The base moiety is included equatorially, as indicated by the practically equal induced chemical shifts

for the base protons (0.50 and 0.46 ppm at $R = 0.78$ for H_5 and H_6 respectively of **2** and 0.85 and 0.80 ppm for H_2 and H_8 respectively of **3**).

In each of the complexes, the nucleotide exists in both the *syn* and *anti* conformations (**4** and **5** respectively for the proposed orientations of binding of guest **2**; for clarity only one vertical naphthalene wall (shaded) of the host is shown and the phosphate moiety behind the sugar ring in **5** is not shown) because the maximum induced chemical shifts of $H_{1'}$ and $H_{2'}$ are practically the same (0.55 and 0.54 ppm for **2** and 0.56 and 0.50 ppm for **3**). In the *syn* conformation, the sugar moiety provides the C—$H_{1'}$ bond for CH-π interaction with the naphthalene wall of the host. In the *anti* conformation it is the C—$H_{2'}$ bond of the sugar moiety. According to our model, there should be no steric interaction between the sugar moiety and the host in the *anti* conformation because the cavity of the host is enclosed by only a pair of antiparallel vertical naphthalene walls while the other pair of opposite naphthalene walls are in a horizontal position [8]. The presence of steric interaction between the sugar moiety and a resorcinol cyclic tetramer host was reported to result in a preferred *syn* conformation for cytidine and cytidine 5′-phosphate [6].



4 **5**

Schemes 4–5.

The stability constant K of each of the two 1 : 1 host to guest complexes was obtained by a nonlinear regression fitting procedure [9]. The K values obtained from different protons of the same nucleotide are in satisfactory agreement with one another (Table I). That the K value for nucleotide **3** is larger than that of **2** is consistent with the presence of more attractive π–π interactions between the guest and host in the former [10].

Acknowledgement

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