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Binding of Nucleotides and Nucleosides to Per(6-guanidino-6-deoxy)-cyclodextrins in Solution

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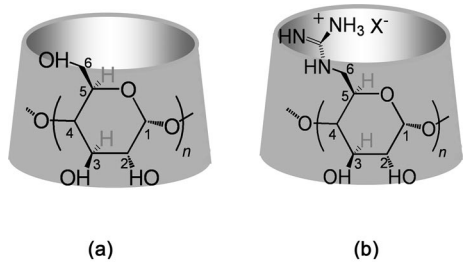
The binding of α-, β-, and γ-per(6-guanidino-6-deoxy)cyclodextrins (ag, bg, and gg, respectively) with nucleotides (5′-dAMP, 5′-AMP, and 5′-dCMP) and nucleosides (2′-dA and 2′-dC) was studied with NMR spectroscopy in aqueous solution. Nucleotides formed strong complexes with bg and gg (not ag) through ditopic binding. Multiple Coulombic attractive forces between the guanidino groups and the single phosphate group of the nucleotides drove inclusion of the entire ribose ring into the cavity, in both deoxy- and ribonucleotides, whereas the base moiety was only partially included. In contrast, no interaction was observed between guanidino cyclodextrins and nucleosides. The prevailing stoichiometries in all nucleotide/bg and nucleotide/gg complexes were 1:2, except for 5′-dAMP/bg where oligomeric aggregates formed. The *anti* orientation of the nucleotides was preserved upon inclusion. The populations of conformers due to ribose pucker, 2′-*endo* (S) and 3′-*endo* (N), were practically undisturbed by inclusion. Interestingly, in the nucleotide/bg complexes at 278 K two nucleotide conformers were observed with distinctly different signals in slow mutual exchange owing to cavity restriction and a slow ribose pucker rate, which resulted in the observation of 3′-*endo* (N) conformer separate from 2′-*endo* (S) conformer for the first time.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharide host molecules having a set of primary hydroxy groups on one side of the macrocyclic structure and a set of secondary hydroxy groups on the other side (Scheme 1a). Introduction of positively charged substituents on the primary side results in derivatives with interesting properties. Per(6-amino-6-deoxy)cyclodextrins[1–5] provide both free amine and ammonium groups at neutral pH and behave as biomimetic catalysts.[6] Previous work in this laboratory showed that per(6-guanidino-6-deoxy)cyclodextrins[7] (Scheme 1b) display very strong ditopic binding towards phosphorylated aryl guest molecules, as opposed to weak cavity binding towards non-phosphorylated aryls.

Furthermore, guanidino CDs interact with DNA and actually compact it into nanoparticles,[7] resulting in its entry inside cells and finally in the expression of the protein that this DNA encodes.[8] The mode of binding to DNA is not known for these CDs or for other CD-based transfection agents modified on the primary side with elaborate positively charged groups.[9–11] The present work is an NMR spectroscopic study of the interactions of guanidino CDs with nucleic acid components, namely, the nucleotides 2′-deoxyadenosine 5′-monophosphate (5′-dAMP), adenosine 5′-monophosphate (5′-AMP), and 2′-deoxycytidine 5′-monophosphate (5′-dCMP) (Scheme 2) and the nucleosides 2′-deoxyadenosine (2′-dA) and 2′-deoxycytidine (2′-dC) (Scheme 2). The conformations of the nucleotides are analyzed in their free forms and in the presence of the guanidino CDs in order to evaluate any binding-induced conformational changes.



Scheme 1. Representation of (a) the α-, β-, and γ-cyclodextrin macrocycles with n = 6, 7, and 8, respectively; (b) guanidinylated analogs ag, bg, and gg.

A review of the literature revealed basic and important work that was presented during the 1970s and 1980s by using 1D NMR experiments. The *syn* ⇌ *anti* equilibrium, referring to the orientation of the nucleobase with respect to the glycosidic bond, and the populations of the 3′-*endo* (N) ⇌ 2′-*endo* (S) conformers, referring to the puckering of the ribose ring in nucleotides (Scheme 3) were studied by various NMR spectroscopic methods in the past. Compari-

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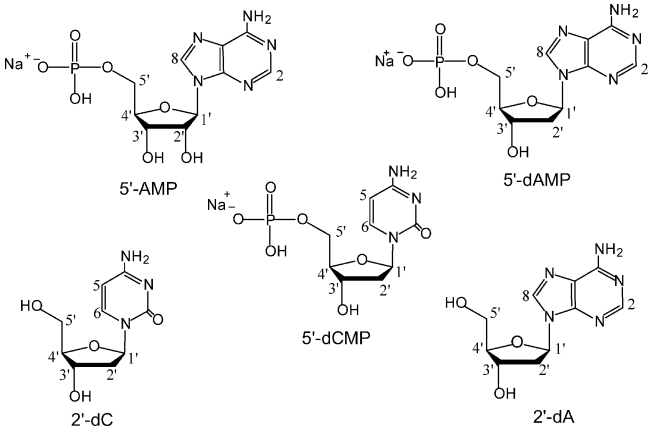
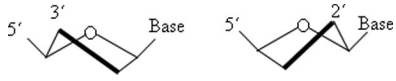


Scheme 2. Nucleotides and nucleosides used drawn in their *anti* conformations.

son of chemical shifts,[12–14] vicinal spin–spin coupling constants,[15] 1D nuclear Overhauser enhancements, and nuclear relaxation rates,[16] as well as the effects of lanthanide cation probes (Ln^{3+})[17] on chemical shift *and* relaxation rates, were analyzed.



Scheme 3. The 3′-*endo* (N) (left) and 2′-*endo* (S) (right) conformations of the nucleotides.

Comparison of chemical shifts indicated a dynamic *syn* ⇌ *anti* equilibrium with a preference for the *anti* conformation (≈70%) in nucleosides, whereas there was a dominance for the *anti* conformation (virtually 100%) in nucleotides. Relaxation methods in general showed that the *syn* ⇌ *anti* equilibrium favors the *syn* conformation for nucleosides and 5′-nucleotides; however, the relaxation method developed by Zens,[18] who employed both ^1H and ^2H T_1 measurements, suggested preference for the *anti* conformation. The lanthanide probe method agreed with the results obtained from the comparison of chemical shifts for all nucleotides except for the guanosines. Concerning the 3′-*endo* (N) ⇌ 2′-*endo* (S) conformation, the above studies showed that the S conformer is favored over the N conformer, and more so in 5′-deoxyribonucleotides than in 5′-ribonucleotides.

Results and Discussion

In the present study, one ribonucleotide (5′-AMP), two deoxyribonucleotides (5′-dCMP, 5′-dAMP), and two deoxyribonucleosides (2′-dC, 2′-dA) having a purine (adenine) or a pyrimidine (cytosine) heterocyclic base (Scheme 2) were examined by NMR spectroscopy at 500 MHz in buffered solutions of potassium chloride/hydrogen chloride or acetic acid/sodium acetate at measured acidities of 2.1 and 5.7, respectively. The corresponding pD values are estimated to be higher by ca. 0.3 units, that is, 2.4 and 6.0, respectively.[19,20] The nucleotides and nucleosides were studied at 278 and 298 K in the concentration range 2.5–10 mM and at pD = 2.4 and 6.0. The 2D NOESY spectra for 5′-dAMP, 2′-dA, 5′-dCMP, and 2′-dC are shown in Figure 1.

The presence of a strong cross-peak between 8-H of adenine and 2′-H, the proton in the ribose ring pointing toward the base (the proton away from the base is denoted 2′′-H), and the absence of a cross-peak between 8-H and 3′-H in 5′-dAMP indicate that the molecule adopts the thermodynamically more stable 2′-*endo* (S) conformation of the ribose ring, although a very small contribution of the 3′-*endo* (N) conformation cannot be ruled out. A stronger cross-peak between 6-H of cytosine with 2′-H, relative to that with 3′-H in both 5′-dCMP and 5′-dC, and a stronger cross-peak between the 8-H of adenine with 2′-H, relative to that of 3′-H in 2′-dA, indicate that the molecules adopt primarily the 2′-*endo* (S) conformation of the deoxyribose ring with a minor contribution of the 3′-*endo* (N) conformation. In the case of 5′-AMP, the cross-peaks between 8-H of the heterocyclic base with 2′-H and 3′-H are of the same intensity, and thus, the equilibrium 2′-*endo* (S) ⇌ 3′-*endo* (N) is verified. A nucleotide is considered[21] as primarily *anti* if its 8-H (6-H) displays a strong NOE with its 2′-H and a weak NOE with its 1′-H. The opposite is ob-

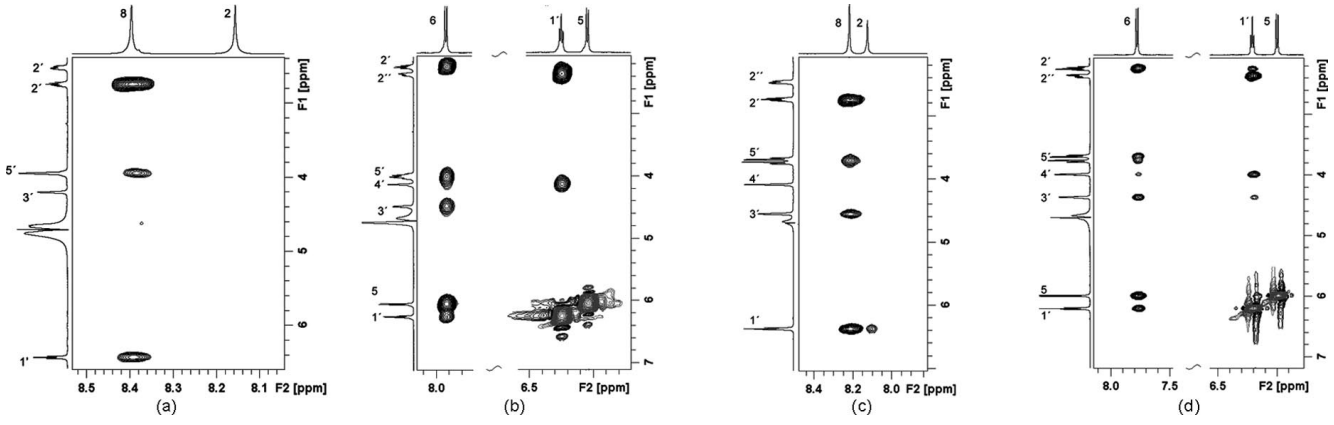


Figure 1. 2D NOESY NMR (500 MHz, D_2O, pD = 6.0, 298 K) spectra of (a) 5′-dAMP (5 mM), (b) 5′-dCMP (5 mM), (c) 2′-dA (5 mM), and (d) 2′-dC (5 mM). Grey cross-peaks appearing in both dimensions in (b) and (d) are parts of the diagonal.

served when the *syn* conformation is preferred. The intramolecular interactions for 5′-dAMP, 5′-AMP, and 2′-dA (Figure 1a,c) revealed strong cross-peaks between 8-H and 2′-H and weaker cross-peaks between 8-H and 1′-H as well as 5′-H, whereas 2-H did not correlate with anything. Similarly, for 5′-dCMP and 2′-dC, the spectra revealed strong cross-peaks of 6-H with 2′-H and weaker cross-peaks with 1′-H, 5′-H, and 3′-H, whereas 5-H did not correlate with anything (Figure 1b,d). Thus, all nucleotides and nucleosides are in the preferred *anti* conformation about the glycosidic bond and all favor the 2′-*endo* (S) conformation. Further, by using Equation (1),[21] the % populations of 3′-*endo* (N) were calculated for 5′-dAMP, 5′-dCMP, and 5′-AMP (Table 1), and the results agreed with the earlier literature values[13,15,17] for the free compounds.

$$\%N = 100(J_{1'2',S} - J_{1'2',obs.})/(J_{1'2',S} - J_{1'2',N}) \quad (1)$$

where $J_{1'2',obs}$ are the observed coupling constants and $J_{1'2'}$ are the published limiting values[22] of the 2′-*endo* (S) and 3′-*endo* (N) conformers.

Table 1. Calculated % of the 3′-*endo* (N) conformer of free nucleotides and in the presence of guanidino CDs.

	Free	Complex with bg	Complex with gg
5′-dAMP	28	32	32
5′-dCMP	29	33	30
5′-AMP	40	Broad signals	≈50

Binding of Guanidine CDs to Nucleotides

Purine nucleotide 5′-monophosphates may accept two protons at their phosphate group and an additional one at the purine moiety. The first deprotonation is characterized by $pK_a = 0.7 \pm 0.3$,[23] although slightly larger values have also been reported[24,25] and occurs at the phosphate group. The second deprotonation with $pK_a = 3.84 \pm 0.02$ occurs at the (*N*7)H^+ adenine site, and the third deprotonation with $pK_a = 6.21 \pm 0.01$ involves loss of a second proton from the phosphate group. For pyrimidine nucleotides and specifically cytosine, the second and third pK_a values are higher, 4.55 ± 0.03 and 6.55 ± 0.02, respectively.[23] Binding of guanidino CDs to nucleotides was examined at pD = 2.4 and 6.0. At pD = 2.4 only the first deprotonation step is complete and *N*7 is deuterated, whereas at pD = 6.0 the base is not deuterated and the phosphate group is ionized. Thus, at pD = 2.4 and 6.0 the guanidinylated hosts and phosphorylated guests are charged positively[8] and negatively, respectively.

5′-dAMP⊂bg

The ^{1}H NMR spectra of 5′-dAMP (5 mM) in the presence of bg (2.5 mM) at pD = 6.0 and 2.4 showed changes in the chemical shifts of all the hydrogen atoms of bg and particularly shielding of the internal CD protons (denoted in italics), *5-H*, *3-H* (0.12 and 0.16 ppm respectively, Table 2), suggesting that bg encapsulates the nucleotide. In addition, in the presence of bg, the sharp peak of 5′-dAMP in the ^{31}P NMR spectrum developed into a shielded, broad peak (Figure 2), indicating the presence of various interactions. Electrostatic guanidinium–phosphate interactions and encapsulation of the nucleotide inside the cyclodextrin cavity were the two possibilities considered.

Table 2. Chemical shift changes for the guanidino CD cavity protons (pD = 6.0).

	$\Delta\delta$*5-H* [ppm]		$\Delta\delta$*3-H* [ppm]	
	bg[a]	gg[a]	bg[a]	gg[a]
5′-dAMP[b]	0.1180	0.0021	0.1661	0.0548
5′-dCMP[b]	0.1032	0.0038	0.1230	0.0305
5′-AMP[b]	0.1543	0.0007	0.1852	0.0329

[a] 2.5 mM. [b] 5 mM.

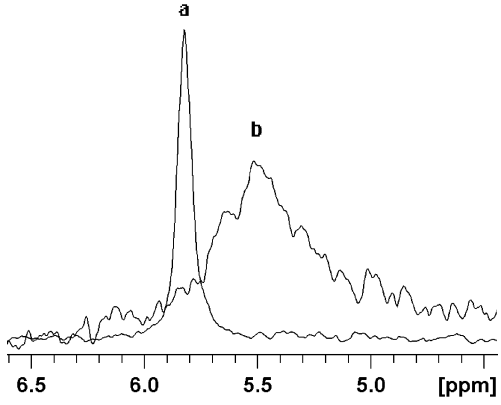


Figure 2. ^{31}P NMR (202.45 MHz, D_2O, pD = 6.0, 298 K) spectrum of (a) 5′-dAMP (5 mM) and (b) 5′-dAMP (5 mM)/bg (2.5 mM).

The ^{1}H NMR signals of 5′-dAMP (Figure 3a) showed broad and overlapped signals in the presence of bg (Figure 3b) at 298 K (at both pD values) that were also affected by the concentrations used and the ratio of 5′-dAMP/bg. A solution of 5′-dAMP (5 mM) and bg (4 mM) was the most advantageous combination of concentrations so that the signals in the ^{1}H spectra were optimally dispersed and easily monitored. The broad peaks resolved adequately at 278 K (Figure 3c). The spectra at this temperature revealed two broad unequal signals for each proton of the nucleotide, denoted as i and ii, at both pD = 2.4 and 6.0 (Figure 3c). In the ^{13}C NMR spectra two unequal signals were similarly observed. Thus, two forms of 5′-dAMP were observed.

The 2D ROESY spectra showed that the double signals of each proton were in mutual exchange, as they displayed cross-peaks of the same phase as the diagonal. In addition, the double signals of 5′-dAMP showed intermolecular NOE interactions with the protons *3-H* and *5-H* of the bg cavity; thus, they both correspond to inclusion complexes and not to free and complexed species in equilibrium (Figure 3d; Table 3).

The above indicated an inclusion equilibrium process involving both forms of 5′-dAMP and, in particular, inclusion of the deoxyribose ring as well as partial inclusion of the purine six-membered ring. Continuous variation (Job's) plots of 5′-dAMP/bg (Figure 4a) exhibit peculiar shapes without maxima. Instead, the linear dependence of

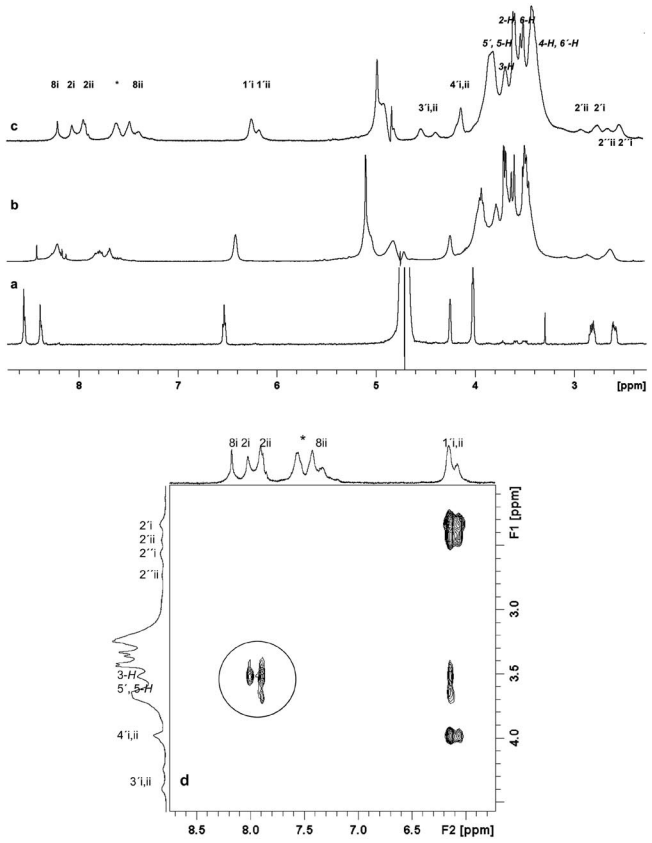


Figure 3. ^{1}H NMR (500 MHz, aqueous buffer, pD = 2.4) of (a) 5′-dAMP (2 mM, 298 K), (b) 5′-dAMP/bg (10 mM each, 298 K), and (c) 5′-dAMP/bg (10 mM each, 278 K); (d) partial 2D ROESY spectrum of 5′-dAMP/bg (both 10 mM, 278 K). Different kinds of 5′-dAMP are denoted as i, ii. The cross-peaks of both i and ii with the cavity protons are circled. *Residual Ph_3PO in bg.

Table 3. Summary of intermolecular guanidino CD/nucleotide interactions.

	Ribose					Base			
3-H, 5-H	1′-H	2′-H	3′-H	4′-H	5′-H	2-H	8-H	5-H	6-H
bg/5′-dAMP	+	+	+	+		+			
gg/5′-dAMP	+	+	+	+		+	+		
bg/5′-dCMP	+							+	+
gg/5′-dCMP	+	+						+	+
bg/5′-AMP	+		+	+		[a]			
gg/5′-AMP	+		+	+		+	+		

[a] Broad signals.

3-H shifts and the almost linear plot of *5-H* suggests that the stoichiometry is not singly defined; this was interpreted as the consequence of oligomeric-type association involving cavity and electrostatic interactions.

The overall experimental results suggest that there are two forms of 5′-dAMP encapsulated in bg, that is, i and ii. Integration of the peaks 3′i-H and 3′ii-H (Figure 3c) gave 69% of form i and 31% of form ii. Analysis of the coupling constants $J_{1'2'}$ according to Equation (1)[21,22] showed that the population of 2′-*endo* (S) is 68% and that of 3′-*endo* (N) is 32%, values that perfectly match the ones obtained by integration of i and ii in the complex. Thus, a reasonable conclusion is that form i is the 2′-*endo* (S) conformer and form ii is the 3′-*endo* (N) conformer, in mutual slow exchange. This can happen because inclusion in the bg cavity is tight, which does not allow fast exchange between the N ⇌ S conformers. The slightly increased population of 3′-*endo* (N) (32% in the complex in comparison to 28% in the free nucleotide, Table 1) is an insignificant change; thus, no preferential stabilization is gained following cavity inclusion. The deoxyribose fits tightly in the cavity with its phosphate group interacting with the guanidino groups of the primary side. Simultaneously, as a result of the angled structure of 5′-dAMP, the adenine moiety can only partially fit into a next bg cavity, leaving space for a second nucleotide to approach from its guanidine side, and so on. The proposed configuration adopted by the complex is shown in Scheme 4a.

5′-dAMPlgg

The shifts of the internal *5-H* and *3-H* signals of gg in the presence of 5′-dAMP were significantly smaller relative to those of bg (Table 2), evidently as a result of the wider cavity of gg. Further, the ^{1}H NMR spectra of 5′-dAMP did not show signs of intermediate or slow equilibrium phenomena at a large range of concentrations and molar ratios. 2D ROESY spectra showed cross-peaks of *3-H* and *5-H* of gg with protons of ribose, as well as adenine moieties (Table 3) of the nucleotide that proved inclusion. Job's plot (Figure 4b) based on gg shifts revealed a maximum close to 0.60; thus, the stoichiometry of 5′-dAMP/gg is close to 1:2, with possible small contribution of 1:1. Simultaneous plotting of 5′-dAMP shifts (Figure 4c) showed contradicting results. This is explainable, as the stacking of nucleotides,[26] being concentration dependent, strongly influences their chemical shifts. We can therefore conclude that the deoxyribose part enters the gg cavity as in bg, but now the larger cavity space allows unrestricted ribose conformational exchange. Thus, slow exchange phenomena are not observed. The % population of each conformer was again calculated from the coupling constants as above (Table 1) and was found to be 32% 3′-*endo*, that is, the same as in bg. The observed stoichiometry, in combination with interactions with both 2-H and 8-H indicates that the adenine ring fits sideways into a second gg host (Scheme 4b).

5′-dAMPlag

The changes in chemical shifts of all ag protons were significantly smaller relative to those of bg. Furthermore, despite the fact that the ^{31}P resonance was remarkably shifted (ΔP = 0.904 ppm), the peak still remained sharp. In addition, the absence of intramolecular interactions in the 2D ROESY spectra proved that only external interactions exist between guanidino and phosphate groups without inclusion of the nucleotide inside the cavity, most likely due to the small size of the host.

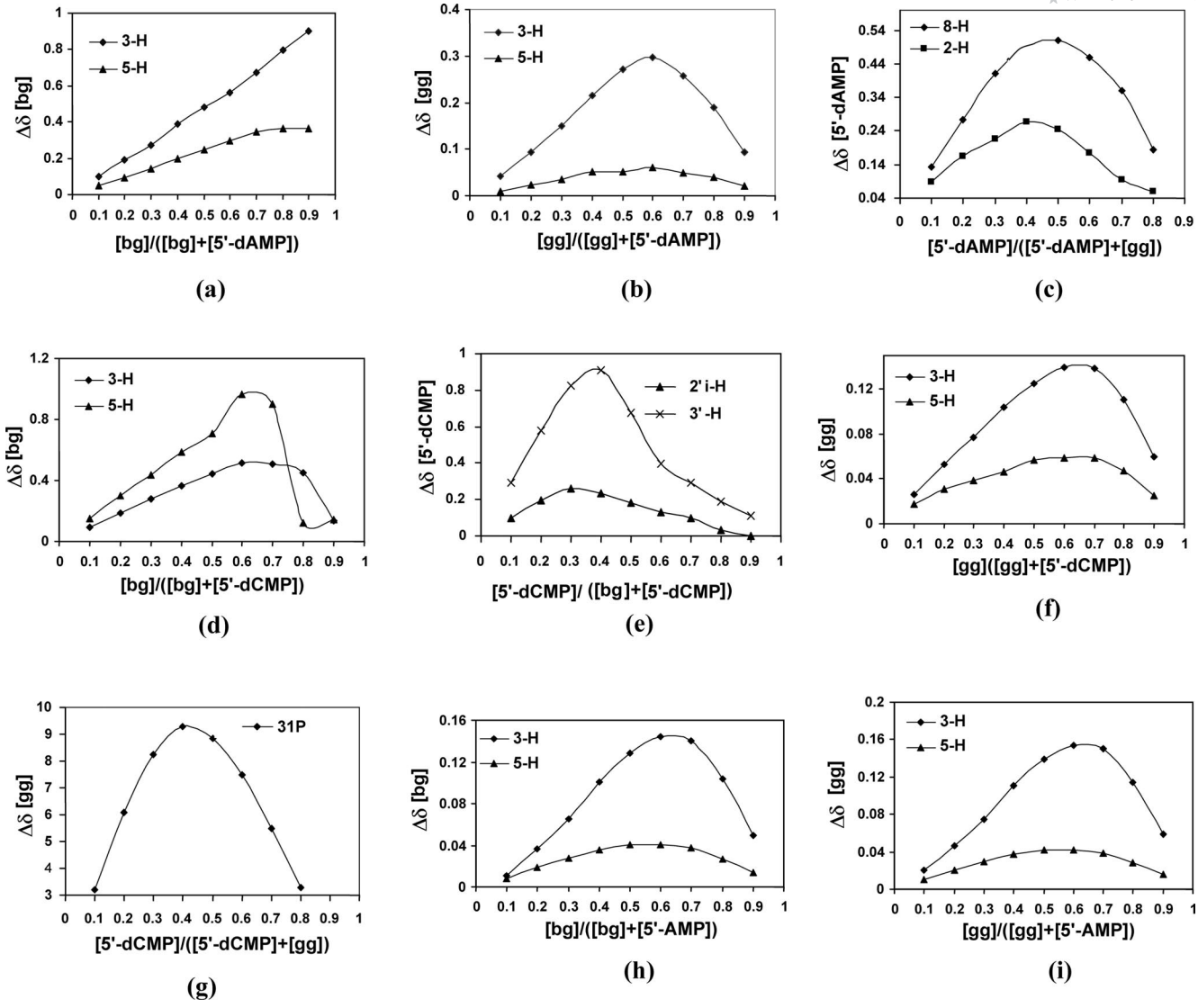


Figure 4. Continuous variation plots of (a) guanidino CDs/nucleotides (500 MHz, pD = 6.0, 298 K).

5′-dCMP/bg

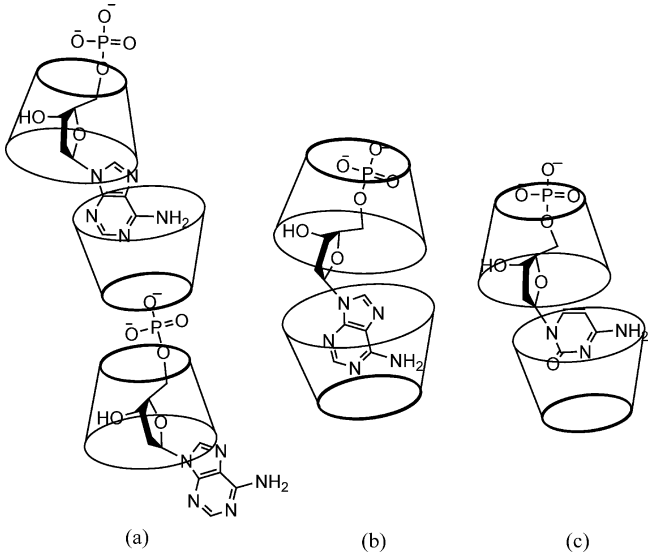
In the presence of bg the peaks of 5′-dCMP showed moderate broadening, which at 278 K gave double resonances, forms i and ii, in some signals. The exchange cross-peaks present in the 2D ROESY spectrum between 2′-H, 3′-H, 4′-H, and 6-H of form i with 2′-H, 3′-H, 4′-H, and 6-H of form ii indicate that two forms of the nucleotide are present, as in 5′-dAMP/bg. Intermolecular NOE cross-peaks of bg *3-H* and *5-H* with 1′-H and with 5-H and 6-H were observed (Table 3). Consequently, 5′-dCMP is included by both cytosine and deoxyribose parts, the latter being in slow exchange between the 3′-*endo* (N) ⇌ 2′-*endo* (S) conformations. According to continuous variation plots with the use of the shifts of bg (Figure 4d) and the normalized shifts of 5′-dCMP (Figure 4e) where the influence of self-association[26] was eliminated by using shifts of blank experiments, the stoichiometry of 5′-dCMP/bg is 1:2. The proposed mode of complexation is shown in Scheme 4c.

5′-dCMP/gg

In the presence of gg, 5′-dCMP showed sharp signals. Protons *3-H* and *5-H* of gg interacted with 5-H, 6-H, 1′-H, and 2′-H (Table 3). Intramolecular interactions of 5′-dCMP revealed cross-peaks of 5′-H with 6-H and 1′-H and of 3′-H with 6-H. Thus, an inclusion complex is formed, whose stoichiometry is again 1:2 (Figure 4f), also verified by plotting the ^{31}P NMR shifts of the nucleotide (Figure 4g). The mode of inclusion is thus similar to that proposed above (Scheme 4c).

5′-AMP/bg,gg,ag

Weak interactions of 1′-H, 3′-H, 4′-H of ribose with *5-H* of bg and of 1′-H with *3-H* of bg suggest weak cavity binding of the ribose ring attributed to the presence of the 2′-OH group that reduces the hydrophobicity of the ribose ring and increases the bulkiness. Adenine 2-H and 8-H signals were broadened and did not show the expected NOE



Scheme 4. Suggested model of the complex between (a) 5′-dAMP/bg, (b) 5′-dAMP/gg, and (c) 5′-dCMP/bg. The thick lines represent the area of the guanidino groups.

interactions with the ribose protons, evidently as a result of an exchange rate regime faster than the timescale of NOE development. Consequently, the absence of cross-peaks with bg cannot be taken as absence of inclusion. The detection of the apparently present- 3′-*endo* (N) and 2′-*endo* (S) conformers is indirect. The gg peaks of 5′-AMP were sharp. Protons 2-H and 8-H interact with *3-H* and *5-H* of gg weakly, whereas 1′-H, 3′-H, and 4′-H interact with the same protons moderately (Table 3). The stoichiometry in both 5′-AMP/bg and 5′-AMP/gg is 1:2 (Figure 4h,i); therefore, the proposed complexation models are similar to those in Scheme 4b,c. Finally, no inclusion complexes were observed for ag with 5′-AMP and also 5′-dCMP.

The results collectively show that both deoxyribonucleotides and ribonucleotides interact with all guanidine CDs through guanidinium–phosphate-type electrostatic interactions. Inclusion complexes, however, are formed with bg and gg and not with ag. With bg broadening of the nucleotide, resonances are observed that at low temperature split into two unequal peaks, attributed to cavity-induced reduction of the ribose pucker rate based on nearly identical *J* coupling analysis and peak integration results. Eliseev and Schneider, who previously studied 5′-dAMP, 5′-AMP, and 5′-dCMP with heptakis(6-deoxy-6-*N*-methylamino)-β-CD,[27] also reported broadening of the peaks; however, that was attributed to *syn* and *anti* conformational exchange on the basis of time-scale considerations. Additionally, they reported partial nucleobase and total ribose inclusion, in agreement with the present results. However, they reported 1:1 stoichiometry on the basis of NMR titration plots, although the continuous variation plots employed here, which are better suited for stoichiometry determination than titration plots, definitely show 1:2 binding or higher. In the same reference[27] potentiometry gave stronger ($K \sim 10^5$ M^{-1}) binding for 5′-AMP than for deoxynucleotides ($K \sim 10^4$ M^{-1}), and this was attributed to H-bonding of the protonated amines to the ribose hydroxy groups. The guanidino groups of our CDs can also establish H-bonds with sugars, as a theoretical study has shown.[28] Thus, we propose here more complex binding models involving oligomeric association for 5′-dAMP/bg and participation of a second cyclodextrin for all other cases. Another potentiometric study has reported strong binding ($K \geq 10^4$ M^{-1}) of 5′-ATP and 5′-AMP with heptakis(6-hydroxyethylamino-6-deoxy)-β-CD[29] and broadening of the host side-chain signals. Recently, ultrasonic relaxation studies of the 5′-AMP/β-CD system showed moderate ($K \sim 10^3$ M^{-1}) binding of the adenine moiety.[30] Lastly, a recent report on bg and its strong inclusion of 5′-ADP/5′-ATP,[31] published shortly after our initial publication on the preparation and properties of ag, bg, and gg,[7] showed doubling of the ATP signals attributed without other evidence to free and complexed species in a (mass spectra inferred) 1:1 complex.

Binding of Guanidino CDs to Nucleosides

2D ROESY or NOESY spectra of 2′-dA as well as 2′-dC in the presence of gCDs and at pD = 6.0 did not show any intermolecular cross-peaks, and no chemical shift changes in the internal CD protons were observed. Consequently, the presence of a phosphate group on the ribose ring is essential for the formation of inclusion complexes. Regarding the conformation of the nucleosides, both molecules were found to be mainly in the 2′-*endo* conformation and in *syn–anti* equilibrium according to the already known bibliographic sources.

Conclusions

In contrast to natural CDs, which totally prefer the nucleobases over the ribose rings and do not stabilize hydroxylated substrates in their cavity, the guanidino CDs as well as other positively charged CDs happily encapsulate both ribose and deoxyribose rings in their cavity, whereas the nucleobase moieties are partially included. The observed tendency does not involve hydrophobic interactions or hydrogen-bond formation.[32] Conversely, it is known[33] that monovalent anionic guests bind stronger to heptakis(6-amino-6-deoxy)-β-CD than to mono(6-amino-6-deoxy)-β-CD. We, in contrast, have shown that phosphorylated aryls bind much more strongly to bg than to bis(guanidinium)-substituted cyclodextrins.[7] Therefore, the stabilizing interactions are accumulative Coulomb forces between guanidino CDs and nucleotides, as we showed that the phosphate groups play an indispensable role in allowing cavity binding driven by the entropic gain acquired from the dehydration of the host and –particularly– the ribose of the guest upon inclusion. Finally, inclusion of nucleotides in the moderately sized bg cavity enabled the observation of separate nucleotide conformers, attributed to slow ribose pucker rates. To the best of our knowledge, this has not been reported before.

Experimental Section

General: Hexakis(6-guanidino-6-deoxy)-α-cyclodextrin hydrochloride (ag), heptakis(6-guanidino-6-deoxy)-β-cyclodextrin hydrochloride (bg), and octakis(6-guanidino-6-deoxy)-γ-cyclodextrin (gg) were prepared as previously described.[7] 5′-AMP and 2′-dA were purchased from Sigma, 5′-dCMP and 2′-dC from Aldrich, 5′-dAMP from Calbiochem, and 99.8% DMSO and 99.9% D_2O from Euriso-top.

Buffered solutions were prepared from sodium acetate/acetic acid (pD = 6.0) and from potassium chloride/hydrochloric acid (pD = 2.4). The pH was measured by using an MP200 Mettler Toledo pH meter. Before each measurement, the electrode system was calibrated in hydrogen ion concentration units p[H] = –log[H^+] by using buffer reference standard solutions (Scharlau) at 298 K. Actually, pD = meter reading + ca. 0.3[19,20] is a reasonable approximation. The temperature of the solutions during the NMR spectroscopic experiments was set at 278 or 298 K.

1D ^1H (250.13 MHz) and ^{13}C (62.90) MHz NMR spectra were acquired with a Bruker AC 250 spectrometer. ^1H (500.13 MHz), ^{31}P (202.46 MHz), and ^{13}C (125.77 MHz), as well as 2D, NMR spectra were acquired with a Bruker Avance DRX 500 spectrometer. ^1H NMR spectra recorded in D_2O were referenced with HOD at δ = 4.79 ppm. 2D ROESY spectra were acquired with presaturation of the residual water resonance and a mixing (spin-lock) time of 350 ms at a field of ca. 2 kHz with the TPPI method by using a 1024 K time domain in F2 (FID resolution 5.87 Hz) and 460 experiments in F1. 2D NOESY spectra were acquired by using mixing times of 1–1.5 s, TPPI detection, and HDO presaturation. Processing was carried out with zero-filling to 1 K in F1 by using sine or qsine (F2) and qsine (F1) window functions, respectively. The experiments were performed at 278 or 298 K in the concentration range 2.5–10 mM and at buffered solutions (potassium chloride/hydrogen chloride, pD = 2.4 or sodium acetate/acetic acid, pD = 6.0). Continuous variation (Job's) plots were constructed by mixing varying volumes of equimolar (either 5 mM or 10 mM) solutions of host and guest compounds to a constant final volume so that the resulting mixtures ranged from 90 to 10% ratio of host–guest equivalents. All experiments were performed at 298 K and buffered at 5.7. The chemical shift differences of the protons of the cyclodextrin host were recorder and plotted with respect to the 1-H signal, a proton external to the CD cavity that undergoes very small changes during complexation.

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[1] R. J. Boger, R. J. Corcoran, J.-M. Lehn, *Helv. Chim. Acta* **1978**, *61*, 2190–2218.
[2] F. Guillo, B. Hamelin, L. Jullien, J. Canceill, J.-M. Lehn, L. De Robertis, H. Driguez, *Bull. Chem. Soc. Fr.* **1995**, *132*, 857–866.
[3] P. R. Ashton, R. Koeniger, J. F. Stoddart, D. Alker, V. D. Harding, *J. Org. Chem.* **1996**, *61*, 903–908.
[4] D. Vizitiu, C. S. Walkinshaw, B. I. Gorin, G. R. J. Thatcher, *J. Org. Chem.* **1997**, *62*, 8760–8766.
[5] W. Tagaki, K. Yano, K. Yamanaka, H. Yamamoto, T. Miyasaka, *Tetrahedron Lett.* **1990**, *31*, 3897–3900.
[6] S. L. Hauser, E. W. Johanson, H. P. Green, P. J. Smith, *Org. Lett.* **2000**, *2*, 3575–3578.
[7] N. Mourtzis, K. Eliadou, C. Aggelidou, V. Sophianopoulou, I. M. Mavridis, K. Yannakopoulou, *Org. Biomol. Chem.* **2007**, *5*, 125–131.
[8] N. Mourtzis, M. Paravatou, I. M. Mavridis, M. L. Roberts, K. Yannakopoulou, *Chem. Eur. J.* **2008**, *14*, 4188–4200.
[9] S.-A. Cryan, A. Holohan, R. Donohueb, R. Darcy, C. M. O'Driscoll, *Eur. J. Pharm. Sci.* **2004**, *21*, 625–633.
[10] A. Dıaz-Moscoso, P. Balbuena, M. Gomez-Garcia, C. Ortiz Mellet, J. Benito, M. L. Le Gourrierec, C. Di Giorgio, P. Vierling, A. Mazzaglia, N. Micali, J. Defaye, J. M. Garcıia Fernandez, *Chem. Commun.* **2008**, 2001–2003.
[11] S. Srinivasachari, K. M. Fichter, T. M. Reineke, *J. Am. Chem. Soc.* **2008**, *130*, 4618–4627.
[12] R. Stolarski, L. Dudycz, D. Shugar, *Eur. J. Biochem.* **1980**, *108*, 111–121.
[13] D. B. Davies, S. S. Danyluk, *Biochemistry* **1975**, *14*, 543–554.
[14] L. Dudycz, R. Stolarski, R. Pless, D. Shugar, *Naturforsch.* **1978**, *34c*, 359–373.
[15] D. B. Davies, S. S. Danyluk, *Biochemistry* **1974**, *13*, 4417–4434.
[16] T.-D. Son, W. Guschlbauer, M. Gueron, *J. Am. Chem. Soc.* **1972**, *94*, 7903–7910.
[17] C. Dobson, M. C. F. G. C. Geraldes, G. Ratcliffe, R. J. P. Williams, *Eur. J. Biochem.* **1978**, *88*, 259–266.
[18] A. P. Zens, T. A. Bryson, R. B. Dunlap, R. Fisher, P. D. Ellis, *J. Am. Chem. Soc.* **1976**, *98*, 7559–7564.
[19] M. Novak, G. A. Bonham, J. J. Mulero, M. Pelecanou, J. N. Zemis, J. M. Buccigross, T. C. Wilson, *J. Am. Chem. Soc.* **1989**, *111*, 4447–4456.
[20] R. C. Weast (Ed.), *CRC Handbook of Chemistry and Physics*, 1st student ed., CRC Press, Boca Raton, Florida, **1988**, p. D-104.
[21] C. Glemarec, M. Jaseja, A. Sandstrom, L. Koole, P. Agback, J. Chattopadhyaya, *Tetrahedron* **1991**, *47*, 3417–3430.
[22] C. Altona, M. Sundaralingam, *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344.
[23] H. Sigel, R. Griesser, *Chem. Soc. Rev.* **2005**, *34*, 875–900.
[24] S. S. Danyluk, F. E. Hruska, *Biochemistry* **1968**, *7*, 1038–1043.
[25] Z. Song, A. Sims, L. Eady, H. Zhao, O. Olubajo, *Can. J. Anal. Sci. Spectrosc.* **2008**, *53*, 45–51.
[26] O. Jardetzky, G. C. K. Roberts, *NMR in Molecular Biology*, Academic Press, New York, **1981**, pp. 187–226.
[27] A. V. Eliseev, H.-J. Schneider, *J. Am. Chem. Soc.* **1994**, *116*, 6081–6088.
[28] C. J. Margulis, *J. Phys. Chem. B* **2005**, *109*, 3639–3647.
[29] P. Schwinte, R. Darcy, F. O'Keeffe, *J. Chem. Soc. Perkin Trans. 2* **1998**, 805–808.
[30] M. Kondo, S. Nishikawa, *J. Phys. Chem. B* **2007**, *111*, 13451–13454.
[31] D.-Q. Yuan, A. Izuka, M. Fukudome, M. V. Rekharsky, Y. Inoue, K. Fujita, *Tetrahedron Lett.* **2007**, *48*, 3479–3483.
[32] H.-J. Schneider, T. Blatter, A. V. Eliseev, V. Riidiger, O. A. Raevsky, *Pure Appl. Chem.* **1993**, *65*, 2329–2334.
[33] K. Kano, T. Kitae, Y. Shimofuri, N. Tanaka, Y. Mineta, *Chem. Eur. J.* **2000**, *6*, 2705–2713.

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