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Inclusion complexes of purine nucleosides with cyclodextrins

II. Investigation of inclusion complex geometry and cavity microenvironment

Tian-xiang Xiang and Bradley D. Anderson

Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84112 (U.S.A.)

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Summary

Inclusion complex formation between purine nucleosides and cycloamyloses (cyclodextrins) has been examined by solubility, circular dichroism, ultraviolet spectrophotometry, and NMR techniques to explore structure-binding relationships, inclusion complex geometry, and the cavity microenvironment. Formation constants of the inclusion complexes were determined by monitoring changes in solubility or circular dichroism spectra with added cyclodextrin. By using various blocking functional groups at different sites in the guest molecules and cyclodextrins varying in size, correlations between cavity size, complex formation constants, and inclusion complex geometry were explored. The effects of complexation on the UV and proton NMR spectra of purine nucleosides were related to the inclusion structure and cavity microenvironment. The rates of isotopic exchange of the H-C(8) hydrogen in adenosine and adenosine arabinoside were measured by NMR spectroscopy at 37 °C and at various concentrations of β-cyclodextrin (β-CD) and hydroxypropyl-β-CD. The marked inhibition of the exchange rates observed upon the formation of inclusion complexes was also related to inclusion structure. All of the data suggest that, in complexes with β-cyclodextrin and its hydroxypropyl derivatives, the purine residue is oriented in the complex with its short axis nearly parallel to the C_7 axis of the β-CD cavity.

Introduction

Cyclodextrins are cyclic oligosaccharides (cycloamyloses) composed of six, seven, or eight glucopyranose units (Schardinger, 1904, 1911). As a result of their torus-like structure, cyclodextrins can act as 'hosts' to include a great variety of molecules of the appropriate size in their cavities (Bender and Komiyama, 1978; Saenger, 1980; Szejtli, 1982). Inclusion complex formation may have an accelerative or decelerative effect on the reactivity of the guest molecule, depending on the nature of the reaction and the orientation of the guest within the cyclodextrin cavity. Cycloheptaamylose (β-CD), for example, has been shown to inhibit completely the basic hydrolysis of ethyl-*p*-aminobenzoate (Lach and Chin, 1964) but to accelerate markedly the hydrolysis of phenyl acetates in alkaline solution in a manner similar to

Correspondence: B.D. Anderson, Department of Pharmaceutics, College of Pharmacy, University of Utah, Salt Lake City, UT 84112, U.S.A.

enzymatic catalysis (Van Etten et al., 1967a,b). These differences in ester reactivity have been ascribed to partial shielding of nucleophilic attack by hydroxide ion in solution in the former example and the close proximity of the ester function to the secondary hydroxyl groups of the cyclodextrin molecule which serve as the 'active site' of the host in the latter. Similar positive or negative catalytic effects of cyclodextrins have been reported for other hydrolytic reactions (Tutt and Schwarz, 1970, 1971; Congdon and Bender, 1971; Brass and Bender, 1973; Mochida et al., 1973), decarboxylations (Cramer and Kampe, 1962, 1965; Straub and Bender, 1972a,b), and oxidation reactions (Cramer, 1953, 1956).

Previous studies of nucleoside-cyclodextrin complexes are limited. Hoffman and Bock (1970) investigated the complex formation between cyclodextrins and nucleic acids as a potential probe of non-helical regions of tRNAs. By UV spectroscopy, only adenine and hypoxanthine derivatives appeared to interact with β-cyclodextrin. Formoso (1973, 1974), however, showed by circular dichroism that 5′-GMP, 5′-UMP, and 5′-CMP also form complexes with β-cyclodextrin. No efforts were made to study the geometry of the inclusion complexes.

Recent studies in these laboratories have focussed on the properties of 2′,3′-dideoxypurine nucleosides, specifically 2′,3′-dideoxyadenosine and 2′,3′-dideoxyinosine, both of which are of interest as potent inhibitors of the reverse transcriptase of the human immunodeficiency virus (HIV) isolated from patients with acquired immunodeficiency syndrome (AIDS) (DeVita et al., 1987). The absence of hydroxyls at the 2′- and 3′-positions dramatically increases the susceptibility of these compounds to acid-catalyzed C–N bond hydrolysis (Anderson et al., 1989). We have recently observed, however, that the acid-catalyzed hydrolysis of 2′,3′-dideoxyadenosine is markedly inhibited by complexation with β-cyclodextrin and hydroxypropyl-β-cyclodextrin (Darrington et al., 1990). Protonation of 2′,3′-dideoxyadenosine, which might be expected to reduce the number of possible energetically favored orientations within the cyclodextrin cavity, was found to decrease significantly the complex formation constant at 25 °C. The extent of inhibition of the reactivity of complexed dideoxyadenosine was approx. 100% for both neutral and protonated complexes. This similarity in the reduction in reactivity for both neutral and protonated forms upon complexation could be rationalized if the transition state, in which the purine residue is protonated at N-7, is destabilized in an inclusion complex.

A more detailed understanding of the relationship between nucleoside structure, inclusion complex formation, and the reactivity of the guest molecule within the cavity requires knowledge of the driving forces for the formation of inclusion complexes between nucleosides and cyclodextrins and the orientation of the guest molecules within the cavity. In an effort to delineate these driving forces and obtain further information on inclusion complex geometry, we have examined the complexation of a variety of purines and purine nucleosides with α- and β-cyclodextrins using both thermodynamic and spectroscopic techniques.

Experimental

Materials and apparatus

Cyclohexaamylose (α-CD), cycloheptaamylose (β-CD), adenine, adenosine, and deuterium oxide (99.8%) were obtained from Aldrich (Milwaukee, WI). 2′-Deoxyadenosine, 5′-deoxyadenosine, 6-(dimethylamino)adenosine, 6-(dimethylamino)adenine, α-D-glucose and adenine arabinoside (Ara-A) were obtained from Sigma (St. Louis, MO). 3′-Deoxyadenosine was purchased from Fluka (Ronkonkoma, NY). 2-Hydroxypropyl-β-cyclodextrins with degrees of substitution (DS) of 2.5 and 5.1 per cyclodextrin molecule (Pitha et al., 1986) were gifts from Dr. J. Pitha, NIH. 2-Hydroxypropyl-β-cyclodextrin with a degree of substitution of seven 2-hydroxypropyl residues per molecule was purchased under the brand name MOLECUSOL from Pharmatec (Alachua, FL). 2′,3′-Dideoxyadenosine with a reported purity of 99% was supplied by the National Cancer Institute.

UV absorption measurements were performed on a Perkin-Elmer, Lambda-7, double-beam

spectrophotometer at room temperature. Proton NMR spectra were obtained on a Varian XL-400 MHz or a J-200 MHz spectrometer at approx. 20 °C. The spectra were referenced to internal HDO and external DSS in D_2O. The assignments for the proton chemical shifts of purine nucleosides are facilitated by the large difference in the spin lattice relaxation times (T_1) between H-C(2) and H-C(8) in the adenine residues and the fact that H-C(8) is readily exchanged for deuterium by heating the compounds in D_2O at approx. 80 °C for a few hours (Schweizer et al., 1964). Circular dichroism spectra were recorded on a Jasco model J-40C automatic recording spectropolarimeter.

Determination of complex formation constants from solubility measurements

An excess quantity of solute was suspended in phosphate buffer (pH 7) containing various concentrations of cyclodextrin in screw-cap vials. The vials were placed in a water bath at specific temperatures and shaken for approx. 2 days. An aliquot was filtered (0.45 μm, ACRO LC3A (Gelman)) and a portion of the filtrate was diluted and analyzed by UV spectrophotometry. Relative solubilities, S/S_0, where S_0 is the solubility of the solute in the absence of cyclodextrins, were plotted as a function of CD concentration [CD]. 1:1 complex formation constants were obtained according to the method of Higuchi and Connors (1965) as described in the following equation:

$$S/S_0 = 1 + \frac{K_{1:1}[\mathrm{CD}]}{1 + K_{1:1}S_0} \qquad (1)$$

Determination of complex formation constants by circular dichroism

Circular dichroism spectra were recorded at various temperatures. The concentrations of purine nucleosides ([C] < 10^{-4} M) were controlled such that the maximum absorbance A, was below 2. Molar ellipticity differences, $\Delta\theta$, were recorded at the wavelength at which the differences were maximized (approx. 260 nm) as a function of cyclodextrin concentration. Complex formation constants were obtained from plots of $1/\Delta\theta$ vs $1/[\mathrm{CD}]$ according to the equation of Benesi and Hildebrand (1949):

$$\frac{1}{\Delta\theta} = \frac{1}{\Delta\theta_{AB}K[\mathrm{CD}]} + \frac{1}{\Delta\theta_{AB}} \qquad (2)$$

where $\Delta\theta_{AB}$ is the difference in molar extinction coefficients for right and left circularly polarized light between the free and complexed purine nucleosides.

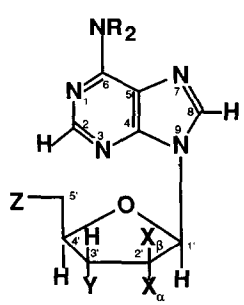
Measurement of isotope exchange kinetics

β-CD and hydroxypropyl β-CD (DS = 2.5) were exchanged for deuterium by drying in vacuo about 100 mg of the respective cyclodextrins in 6 ml of D_2O at least three times. A buffer stock solution of pD 7.10 was prepared by dissolving deuterated sodium hydroxide and phosphoric acid in D_2O. An accurately weighed amount of purine nucleoside was dissolved in this solution. Aliquots of samples were transferred to several vials with different concentrations of β-CD or hydroxypropyl β-CD (DS = 2.5). The samples were sealed and placed in an incubator at 37 °C. Samples were taken out at various time intervals and their proton NMR spectra were taken.

Results and Discussion

Thermodynamic studies of inclusion complex formation

A list of the complex formation constants for various purine nucleosides is shown in Table 1. A general structure for the adenine nucleosides in Table 1 is shown below.



General structure of adenine nucleosides.

TABLE 1

Complex formation constants for various purines and purine nucleosides with cyclodextrins at 25 °C

Substrate [a]	R	X_α	X_β	Y	Z	$K_{1:1}$ (M^{-1})		
						α-CD	β-CD	Hydroxypropyl-β-CD
Adenine	H	NA [b]	NA	NA	NA	3.2(0.5) [c,d]	14.0(1.0) [d]	10.5(1.4) [d,e]
6-(Dimethylamino)-adenine	CH_3	NA	NA	NA	NA	–	31.4(5.9) [f]	–
Adenosine	H	OH	H	OH	OH	< 0.8	13.3(1.0) [d]	12.5(1.5) [d,g]
2′-Deoxyadenosine	H	H	H	OH	OH	–	25.1(5.8) [d]	33.2(6.2) [d,e]
3′-Deoxyadenosine	H	OH	H	H	OH	–	25.2(6.5) [f]	–
5′-Deoxyadenosine	H	OH	H	OH	H	–	21.9(1.5) [f]	22.2(2.3) [f,g]
2′,3′-Dideoxy-adenosine (neutral)	H	H	H	H	OH	–	29.2(10.6) [f]	30.9(6.1) [f,e] 47.6(2.6) [h,i]
2′,3′-Dideoxy-adenosine (protonated)	H	H	H	H	OH	–	–	12.3(0.4) [h,i]
Ara-A	H	H	OH	OH	OH	< 0.4	42.4(5.4) [d]	50.1(8.2) [d,e]
6-(Dimethylamino)-adenosine	CH_3	OH	H	OH	OH	–	128.6(10.5) [f]	–

[a] Refer to general structure of adenine nucleosides. [b] Not applicable. [c] Numbers in parentheses are standard deviations. [d] Measured by solubility method. [e] Degree of substitution in hydroxypropyl-β-CD = 2.5. [f] Measured by circular dichroism. [g] Degree of substitution in hydroxypropyl-β-CD = 5.1. [h] From Xiang and Anderson (1989). [i] Degree of substitution in hydroxypropyl-β-CD = 7.

Cyclodextrins have been shown to have a toroidal, hollow, truncated cone structure with cavities of specific sizes. The extent of inclusion depends critically on the size of the guest species relative to the dimensions of the host cavity. The wider edge of the α-CD cavity has a diameter of 5.7–6 Å (Cramer et al., 1967; Szejtli, 1982). The dimensions of adenine, taking into consideration the Van der Waals radii of the aromatic hydrogens and the $-NH_2$ group at the C(6) position, are estimated from X-ray studies of purines (Broomhead, 1948; Cochran, 1951; Pauling and Corey, 1956; Spencer, 1959) to be 7 Å from N(3) to the $-NH_2$ group at C(6); 5.9 Å from N(7) to

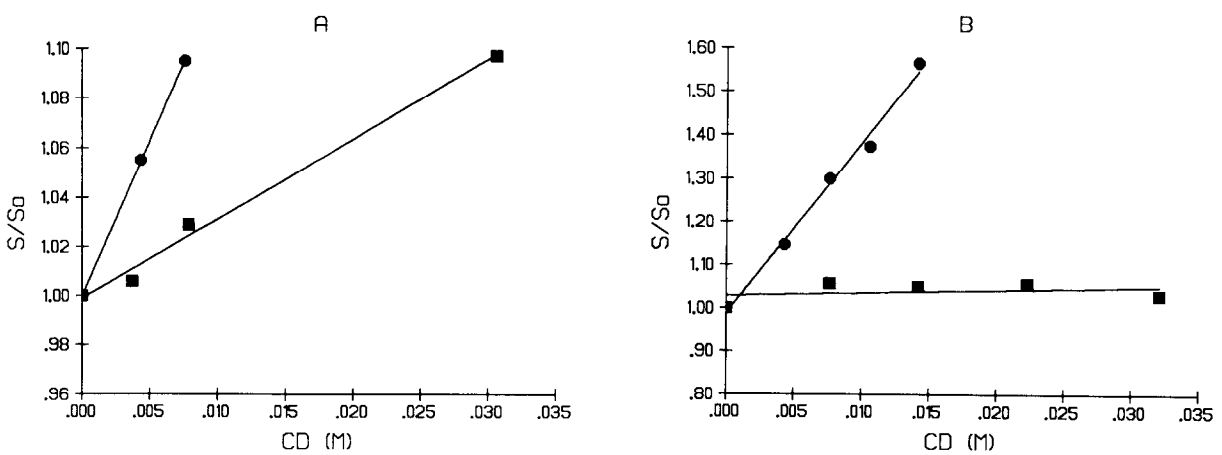
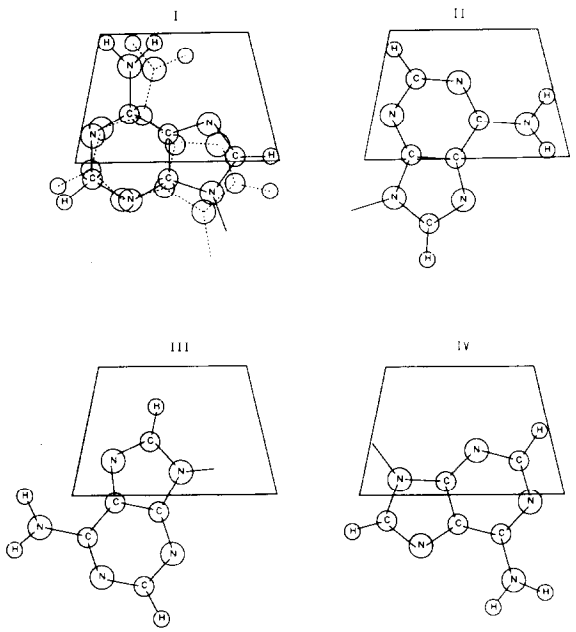


Fig. 1. Solubility plots of adenine (A) and Ara-A (B) vs concentration of added α-cyclodextrin (■) or β-cyclodextrin (●).



Scheme 1. Inclusion structures I–IV.

the N(9) proton; 6.7 Å from N(1) to N(7); 8.2 Å from N(1) to the C(8) proton; and 8.9 Å along the longest dimension from HC(2) to HC(8). As a result, for inclusion complexes of adenine with α-CD, the only possible site for the inclusion is at the imidazole portion which has an estimated length of 5.9 Å. The penetration of adenine into the cavity is expected to be limited, since the pyrimidine portion, with a $-NH_2$ at the C(6) position, cannot fit into the cavity. As a result, the interaction between α-CD and adenine should be very weak. This is reflected in the small binding constant, $K_f = 3.2\ M^{-1}$, as shown in Fig. 1A and Table 1. It is expected that a large substituent, such as a furanosyl at N-9 of the imidazole residue, should completely block the access of adenine into the cavity of α-CD. Experimentally, this is reflected in the absence of any detectable amount of complexation as illustrated in Fig. 1B for adenosine arabinoside (Ara-A).

The diameter of the cavity in β-CD is 7.5–7.8 Å (Cramer et al., 1967; Szejtli, 1982). Therefore, four inclusion structures, I–IV, shown in Scheme 1, may be considered. In structure I, a slight tilt of the guest molecule in a clockwise direction (structure Ia) seems to give the best fit to the β-CD cavity, and may be still more pronounced in the case of the $-N(CH_3)_2$ substituted compounds. The substitution of a furanosyl for hydrogen at the N(9) position such as in adenosine eliminates the possible existence of structures III and IV based on the molecular dimensions. In the case of 6-(dimethylamino)adenosine, two methyl substituents greatly increase the size of the adenine residue along the N(3)–C(6) axis (estimated to be 9.0 Å) and should completely block the entrance of the adenine ring into the cavity of β-CD according to structure II. If structure II is the energetically favored inclusion structure for complexes of purine nucleosides with β-CD, a decrease in the complex formation constant upon *N*-methylation would be expected. However, the opposite result was observed. The binding constant for the complex of 6-(dimethylamino)-adenosine with β-CD is about one order of magnitude greater than that of adenosine with β-CD as seen in Table 1. This result can be most easily rationalized if structure I dominates, since in this structure the two methyl substituents will not block the access of the adenine residue into the cavity but instead will enhance its hydrophobic interaction with β-CD.

Also listed in Table 1 are the complex formation constants of the various purine nucleosides with hydroxypropyl-β-cyclodextrin having various degrees of substitution (Pitha et al., 1986). Hydroxyalkylated derivatives of β-cyclodextrin and their complexes are highly water soluble, whereas the solubility of β-cyclodextrin is limited (1.8 g/100 ml (French et al., 1949; Wiedenhof et al., 1968)). The higher aqueous solubility of the hydroxypropyl derivatives was an advantage in some studies as it allowed experiments at higher CD concentration. Table 1 shows that the binding constants of purine nucleosides are not significantly altered by chemical modification of the primary hydroxyls to form the hydroxypropyl derivatives.

Complex formation constants for β-CD/Ara-A and adenosine complexes were also measured as a function of temperature between 5 and 50 °C. Standard enthalpy and entropy changes were calculated according to the van't Hoff equation, $d \ln(K_f)/d(1/T) = -\Delta H_f^0/R$, and are listed with

TABLE 2

Thermodynamic parameters in inclusion complex formation

Substrate	ΔH° (kcal/mol)	ΔS° (e.u.)	ΔG° at 25 °C (kcal/mol)
Ara-A	−6.8	−15.3	−2.2
Adenosine	−5.1	−12.7	−1.3
2′,3′-Dideoxy-adenosine (neutral) [a]	−2.8	−2.0	−2.3
2′,3′-Dideoxy-adenosine (protonated) [a]	−2.5	−3.5	−1.5
Indole [b]	−4.1	−3.5	−3.1

[a] From Darrington et al. (1990). [b] Orstan and Ross (1987).

the standard free energy changes at 25 °C in Table 2. Also listed are data reported previously for complexes formed with indole (Orstan and Rass, 1987) and with 2′,3′-dideoxyadenosine in neutral and protonated forms (Darrington et al., 1990). More favorable interaction enthalpies appear to be largely compensated by the negative entropies which may arise from the substantial loss in the translational and rotational degrees of freedom for those guest molecules which are more tightly bound in the complexes. It has been pointed out previously (Lewis and Hansen, 1973) that cyclodextrins are not very discriminating hosts in water because of this compensation effect.

Complexation effect on UV spectroscopic properties

The 1 : 1 stoichiometry of complexes of β-CD with purine nucleosides is suggested by the UV absorption spectra. Isosbestic points are observed in the UV spectra of β-CD/purine nucleoside complexes consistent with the assumption that only two UV absorbing species exist. One example of the UV spectra, that for Ara-A, is shown in Fig. 2. The possibility that the UV spectral changes observed are a solvent effect caused by the addition of β-CD in the aqueous solution was examined by measuring the effect of D-glucose on the UV spectra of purine nucleosides. At the same w/v concentration, only approx. 10% of the total change in intensity could be accounted for by a solvent effect. Therefore, the induced changes in absorbance are attributed primarily to the formation of inclusion complexes. In Fig. 2A, significant decreases in peak intensity are noted in the 205 and 260 nm bands with complexation. Fig. 2B shows the absorbance difference spectrum in which two very weak positive maxima (expanded × 10) and two negative maxima are found. These altered spectra are assumed to result from changes in the solvent microenvironment upon inclusion of the solute. Gratzer and McClare (1967) have studied the effect of addition of hydrogen-bonding solvents (acetic acid and ethanol) on the absorption of 9-ethyladenine dissolved in chloroform or isooctane. The 260 nm absorption band of 9-ethyladenine is decreased with a decrease in the concentration of ethanol or acetic acid in chloroform or isooctane solution. This suggests that the observed reduction of the peak intensity of the 260 nm band upon the addition of β-CD may result from the loss of hydrogen bonding (probably at

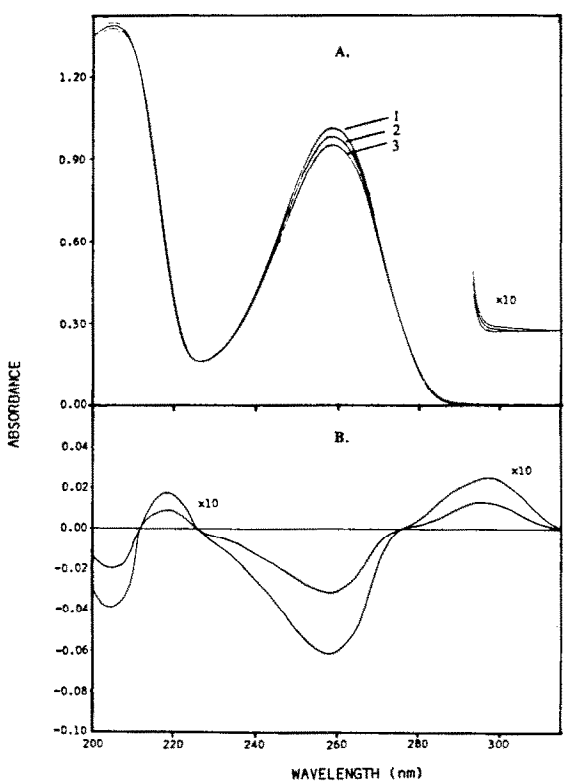


Fig. 2. Effect of β-CD on the UV spectrum of Ara-A in pH 7.10 phosphate buffer. (A) UV absorption spectrum of 4.2×10^{-5} M Ara-A in the absence of β-CD (1) and with β-CD present at 0.0053 M (2) and 0.016 M (3). (B) Spectra in panel A plotted in the form of absorption difference spectra.

sites N(2) and N(7)) accompanying the transfer of the guest molecule from water to the cyclodextrin cavity. This is reasonable in light of the fact that there are no proton-donating functional groups in the cavity of the cyclodextrin molecule.

Complexation effect on proton NMR spectroscopic properties

Fig. 3 shows the effect of increasing the molar ratio of cyclodextrin : purine nucleoside upon the ^1H chemical shifts of the H-C(2) and H-C(8) protons in Ara-A, adenosine and 2′-deoxyadenosine. In this study, hydroxypropyl-β-CD (DS = 2.5) was used rather than β-CD because of its much higher aqueous solubility and similar complexing capability. The solvent effect on the chemical shifts as a result of introducing cyclodextrin was tested by examining the effect of varying concentrations of D-glucose. A very small downfield shift was generally observed (approx. 0.004 ppm at 0.3 M^{-1}). While complex formation induces very small changes in the resonances of the H-C(2) and H-C(8) protons in 2′-deoxyadenosine, adenosine and in the H-C(2) proton of Ara-A, a large upfield shift occurs in the chemical shift of H-C(8) in Ara-A. If the inclusion complexes for adenine nucleosides adopt structure II in which

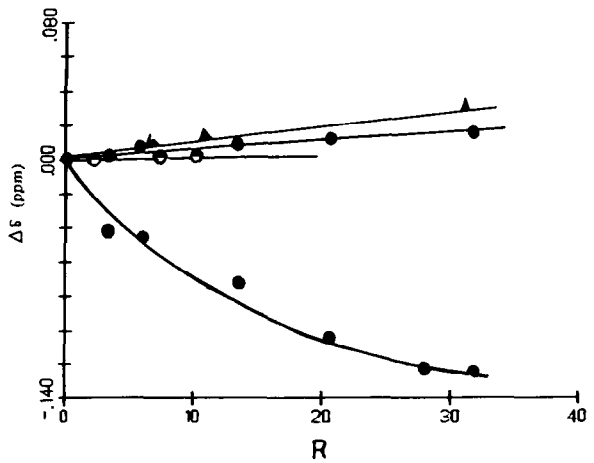


Fig. 3. Cyclodextrin-induced chemical shift changes of the H-C(2) and H-C(8) protons in the adenine residues of adenine nucleosides. (▲) 2×10^{-3} M adenosine, ◓ 2×10^{-3} M 2′-deoxyadenosine, (●) 2×10^{-3} M Ara-A (H-C(2), upper curve; H-C(8), lower curve). *R*: molar ratio of hydroxypropyl-β-CD (DS = 2.5) to the guest.

the H-C(8) proton is located outside the cavity, its chemical shift should move slightly downfield as a result of the medium effect as observed in D-glucose solution. Since this does not occur, the results in Fig. 3 can be best rationalized if the inclusion structure I dominates. In this structure, the induced shifts will strongly depend on the extent of penetration of the adenine residue into the cyclodextrin cavity. A calculation of the dimension of adenine indicates that the length along the long axis of the adenine residue including the two protons on C(2) and C(8) is 8.9 Å – larger than the diameter of the β-CD cavity. As a result, in structure I the two protons in adenine cannot fit into the cavity without a substantial distortion of the cavity. However, H-C(8) alone may penetrate into the cavity.

The large upfield shift of H-C(8) in Ara-A can be well explained by the dramatic microenvironmental change around H-C(8) if the proton is included in the β-CD cavity. The interior lining of the CD cavity is composed of –CH– units and glycosidic bridge oxygens. As a result, the microenvironment of the interior, in the absence of water, may be expected to be similar to that of dioxane. This is consistent with observations (VanEtten et al., 1967b) that the UV spectral changes of some compounds upon cyclodextrin complexation are almost identical with those observed when the compounds are dissolved in dioxane solvent. Hruska et al. (1968) have also shown that a large upfield shift of the H-C(8) signal occurs with an increase in the concentration of dioxane in dioxane-D_2O mixtures, presumably due to changes in hydrogen bonding. Based on these observations, we have studied the medium effect of dioxane on the NMR spectra of Ara-A. As shown in Fig. 4, large upfield shifts for H-C(8) and H-C(2) result with an increase in the w/w concentration of dioxane in dioxane-D_2O mixtures. The changes for H-C(8) approximately parallel those induced by CD inclusion complexation, whereas the changes for H-C(2) are in the opposite direction. This is consistent with our conclusion that the H-C(8) proton of Ara-A is located inside the cavity, while the H-C(2) proton is not. The relative constancy of the H-C(2) and H-C(8) chemical shifts in 2′-deoxyadenosine and

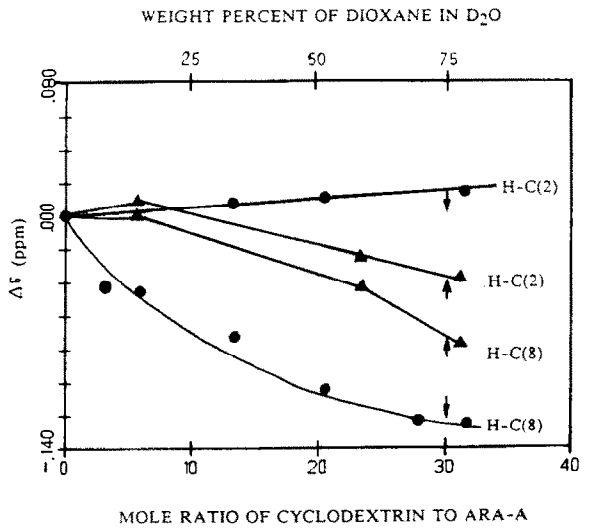


Fig. 4. Comparison between the chemical shifts in 2×10^{-3} M Ara-A induced by hydroxypropyl-β-cyclodextrin (DS = 2.5) inclusion complex formation (●) and those induced by the medium effect of added dioxane (▲). Arrows indicate the x-axis applicable to each curve.

adenosine and that of H-C(2) in Ara-A on complexation suggests that these protons are located outside the inclusion cavity. The deeper penetration of Ara-A into the β-CD cavity, as supported by the chemical shift of the H-C(8) signal, may be attributed to an interaction of the –OH at position C(2′) in Ara-A with β-CD. Inspection of Table 1 shows that Ara-A has a complex formation constant of 42 M^{-1}, about 2- and 3-fold greater than the formation constants for 2′-deoxyadenosine and adenosine, respectively. Table 2 also shows a stronger enthalpy and less favorable entropy of binding for Ara-A compared to adenosine, consistent with tighter binding of Ara-A. These three molecules have similar molecular composition and structure, the only differences being at the C(2′) position. The –OH substituent at C(2′) in Ara-A is above the furanosyl plane where it may play a role in enhancing inclusion complex formation through hydrogen bonding with a secondary hydroxyl group crowning the wider rim of the β-CD cavity.

Isotopic exchange as a probe of local inclusion structure

Measurements of the rates of isotope exchange provide information about the dynamic accessibility of sites within molecular systems. This method has been successfully used to evaluate the mobility of protein molecules in solution (Gurd and Rothgeb, 1979). This technique is used in our present research to study the local structure of the inclusion complex by detecting the relative isotopic exchange rates of the H-C(8) proton in Ara-A and adenosine as a function of cyclodextrin concentration. It is assumed that if a site of isotopic exchange is included in a host cavity, steric hindrance to the access of solvent molecules will lead to a detectable inhibition of the rates of isotopic exchange.

Fig. 5 shows representative semilogarithmic plots of C_t/C_0 versus time, where C_t and C_0 are the concentrations of non-deuterated nucleoside at time t and initial time, respectively. The concentration is expressed in terms of relative peak intensity, I(H-8)/I(H-2), where I(H-2) is used as an internal standard, since the exchange rate of proton H-C(2) is negligible. A dramatic inhibition of the exchange rate in the presence of β-CD and hydroxypropyl-β-CD is noted from these plots. No detectable change in the isotopic exchange rate was observed when 0.20 M D-glucose was added instead of β-CD. The slopes of the solid lines in Fig. 5 determined from least-squares regression represent the pseudo-first-order con-

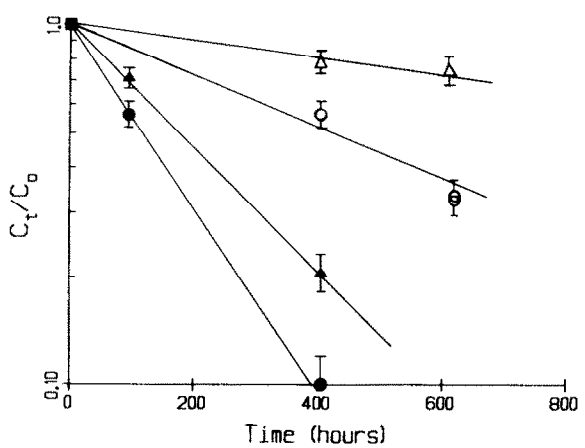


Fig. 5. Relative concentrations of the non-deuterated H-C(8) forms of Ara-A (2×10^{-3} M, open symbols) and adenosine (2×10^{-3} M, closed symbols) vs time in deuterated pD 7.10 buffer. (●,○) No cyclodextrin present; (▲,△) in the presence of 0.063 M hydroxypropyl-β-CD (DS = 2.5).

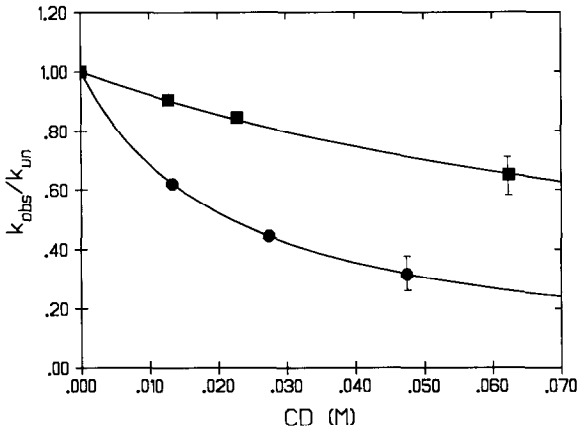


Fig. 6. Plots of the ratios of the observed first-order exchange rate constants in solutions containing hydroxypropyl-β-cyclodextrin (DS = 2.5) to the rate constants in the absence of hydroxypropyl-β-CD vs CD concentration at 37 °C. (■) Adenosine, (●) Ara-A.

stants which are plotted in Fig. 6 as a function of CD concentration. If two species (complexed and free) are assumed to exist at any given CD concentration, as depicted below,

$$\begin{array}{ccc} \text{S-H} + \beta\text{-CD} & \overset{K_f}{\rightleftharpoons} & \text{S-H} \cdot \beta\text{-CD} \\ {\scriptstyle D_2O}\downarrow{\scriptstyle k_{un}} & & {\scriptstyle D_2O}\downarrow{\scriptstyle k_c} \\ \text{S-D} + \beta\text{-CD} & \overset{K_f'}{\rightleftharpoons} & \text{S-D} \cdot \beta\text{-CD} \end{array}$$

then the following rate equation is derived,

$$k_{obs}/k_{un} = \frac{1 + k_c K_f [CD]/k_{un}}{1 + K_f [CD]} \qquad (3)$$

The evaluated parameters obtained from least-squares regression on the data in Fig. 6 using the above equation are listed in Table 3. The observed inhibition efficiencies, k_c/k_{un}, for Ara-A and adenosine may have a bearing on the inhibition mechanism and the inclusion structures. Whereas the NMR chemical shift data suggest that the H-C(8) proton of Ara-A lies within the cavity and that of adenosine appears to lie outside the cavity, inclusion complex formation inhibits the isotopic exchange rate of the H-C(8) proton in both compounds, although the inhibition efficiency is higher (smaller k_c/k_{un}) for Ara-A. This apparent inconsistency may be rationalized by considering the isotope exchange mechanism (Shelton and Clark, 1967; Tomasz et al., 1972; Elvidge et al., 1973), shown in Scheme 2.

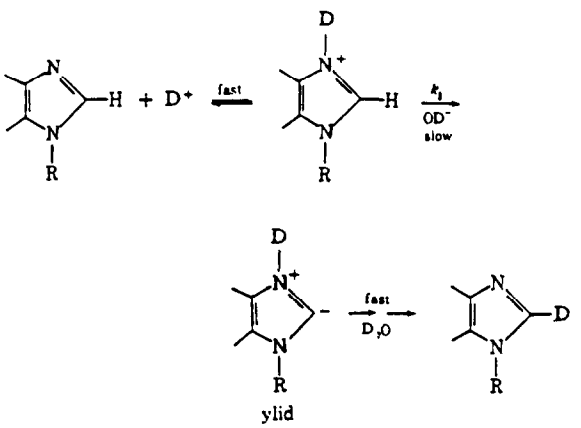
TABLE 3

Parameters obtained from isotopic exchange kinetic study at 37 °C

Substrate	k_c/k_{un}	K_f	K_f [a]
Ara-A	0.006(0.002) [b]	46.4(0.2)	27.1(2.0)
Adenosine	0.0(ND [c])	8.5(0.1)	8.9(0.6)

[a] Obtained from solubility method at 37 °C. [b] Data in parentheses are standard deviations. [c] Not determined.

As neutral pH, the first step of the reaction is the protonation of the N-7 heteroatom in the imidazole ring. It is then followed by a rate-determining abstraction of H-C(8) by OD^-. The generated ylid type intermediate is quickly reprotonated by D_2O. For $K_a \gg [H^+]$, the mechanism predicts the first-order rate constant, $k_{obs} = k_1 K_w/K_a$, where K_a is the dissociation constant for the N-7 protonated purine, k_1 being the rate constant for the rate-determining step. The inhibition of the exchange rate upon the formation of the inclusion complexes may be a result of the increase of the dissociation constant for the protonated N-7 in the cyclodextrin cavity, protection of H-C(8) from attack by OD^- or both. However, our results show that the isotopic exchange reactions of both Ara-A and adenosine are inhibited upon the formation of inclusion complexes even



Scheme 2. Isotopic exchange mechanism.

though the NMR chemical shift data suggest that the H-C(8) proton in Ara-A is located inside the β-CD cavity whereas the same proton in adenosine resides outside the cavity. The result can be rationalized only if the first step in the exchange reaction, the protonation of N-7, is greatly affected. Namely, inclusion complex formation increases the acid dissociation constant, K_a. K_a would be expected to increase in the included structure due to the decreased relative permittivity of the cavity and the steric hindrance to the entry of water molecules which play a greater role in the stabilization of the protonated species than the neutral form (Kondo et al., 1976). Similar arguments may account for the dramatic stabilization of dideoxypurine nucleosides toward acid-catalyzed C–N bond hydrolysis upon formation of inclusion complexes with cyclodextrins (Darrington et al., 1990), even though the C–N bond or the oxocarbonium ion formed in the transition state (Garrett and Mehta, 1972; Romero et al., 1978; York, 1981; Anderson et al., 1989) may not be inside the cavity. In this reaction, also, protonation at N-7 is believed to be on the reaction path (Alivisatos et al., 1962; Holmes and Robins, 1965).

In conclusion, the above results suggest that the microenvironment around the N-7 site of adenine nucleosides is dramatically altered upon the formation of inclusion complexes. We have also shown that the H-C(2) proton lies outside the cavity while the H-C(8) proton appears to reside inside the cavity for tightly bound complexes but outside the cavity with looser binding. In structure II, the outer edge of the imidazole ring of the adenine residue is located outside the wider rim of the cavity. This is inconsistent with the NMR chemical shift data and the isotopic exchange rates. Structure IV is inconsistent with the H-C(2) chemical shift data. Structure III, which was inconsistent with the larger binding constants observed upon methylation of the 6-amino substituent in adenine and adenosine, is also incompatible with the observation that the H-C(8) proton of adenosine lies outside the cavity. These results, therefore, suggest that the binding depicted in structure I is preferred for adenine nucleosides in their neutral form.

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