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Inclusion complexes of purine nucleosides with cyclodextrins

I. Complexation and stabilization of a dideoxypurine nucleoside with 2-hydroxypropyl-β-cyclodextrin

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Summary

2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) markedly inhibits the acid-catalyzed hydrolysis of 2′,3′-dideoxyadenosine (DDA), a compound under development for the treatment of HIV infections. The kinetic data conform to a model which assumes 1 : 1 complex formation between HP-β-CD and both the neutral and protonated forms of DDA. UV difference spectra were generated as a function of both HP-β-CD concentration and temperature to obtain independent estimates of formation constants and enthalpies and entropies for the binding of the neutral species of DDA to HP-β-CD. The acid lability of DDA at the low pH necessary to form the protonated species precluded the use of difference spectra to obtain complexation data for the protonated species. Binding constants for the protonated species were therefore obtained by combining apparent pK_a values as a function of HP-β-CD concentration and temperature with the spectral data. Binding constants for the neutral species ranged from 28 M^{-1} at 50 °C to 56 M^{-1} at 4 °C and were at least 3-times larger than those of the protonated species over this temperature range. A negligible difference in the enthalpy of complexation was observed between the protonated and neutral forms of DDA, while the entropy of formation appeared to favor the neutral complex. Although hydrolysis is 100% suppressed in both the protonated and neutral complexes, due to the small binding constants, the maximum stabilization attainable in a 0.1 M solution of HP-β-CD at 25 °C was approx. 5-fold at pH 5 and 2-fold at pH 2. Possible inclusion geometries are considered in an attempt to account for the kinetic data.

Introduction

β-Cyclodextrins (β-CDs) are able to form inclusion complexes with a number of molecules having a size compatible with the dimensions of the cyclodextrin cavity (Szejtli, 1982). From the standpoint of reaction kinetics, inclusion complex formation may result in increases or decreases in guest reactivity depending on the nature of the reaction and the orientation of the guest molecule within the cavity. This unique catalytic (positive and negative) feature has been used as a model for enzyme-substrate interactions (Tabushi, 1982), to enhance stereoselectivity of chemical reactions (Vanetten et al., 1966), and to improve molecular stability in condensed phases (Uekama et al., 1979). Several reviews on these topics are available

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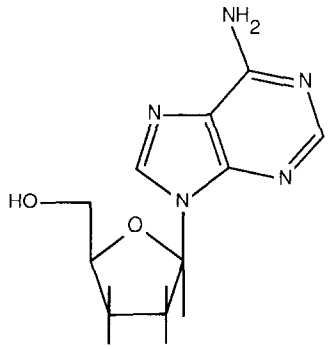


Fig. 1. Structure of 2′,3′-dideoxyadenosine.

(Griffiths and Bender, 1973; Bender and Komiyama, 1978; Jones et al., 1984). However, a complete understanding of the mechanisms of formation of inclusion complexes and their relation to reaction kinetics and energies of complex formation has not yet been achieved.

Certain dideoxynucleosides such as 2′,3′-dideoxyadenosine (DDA) (Fig. 1) are known to be potent inhibitors of the reverse transcriptase of the human immunodeficiency virus (HIV) isolated from patients with acquired immunodeficiency syndrome (AIDS) (DeVita, 1987). Previous studies (Anderson et al., 1988) have shown that the acid lability of such nucleosides is significantly increased upon removal of the 2′,3′-hydroxyls, causing the solution stability of dideoxynucleosides to be a significant concern in dosage form development. Moreover, the low solubility of some dideoxynucleosides may also pose problems, particularly in the development of solution dosage forms. Methods for improving the acid stability and solubility of dideoxynucleosides may therefore have practical utility as well as theoretical importance.

The focus of the present work is the effect of complexation on the acid-catalyzed hydrolysis of DDA, a model dideoxypurine nucleoside, and the relationships between the state of ionization of DDA, the energies of complex formation, and the reactivity of DDA within complexes. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) was chosen over β-CD as the model cyclodextrin for these studies due to its much greater water solubility compared to that of β-CD, so that studies could be extended to higher concentrations (Yoshida et al., 1988). As a result of this higher water solubility, HP-β-CD has also been shown to be less toxic than β-CD (Yoshida et al., 1988). Preliminary studies in these laboratories suggested that complex formation constants are similar for HP-β-CD and β-CD.

Materials and Methods

DDA with a reported purity of 99% was supplied by the National Cancer Institute (Bethesda, MD). Adenine was purchased from Aldrich (Milwaukee, WI). HP-β-CD with a molecular weight of 1540 (corresponding to a degree of substitution of 7, 2-hydroxypropyl residues per molecule) was purchased under the brand name MOLECUSOL from Pharmatec (Alachua, FL). All other compounds were reagent grade from commercial sources and were used without further purification.

HPLC analyses

Hydrolysis rates were monitored by high-performance liquid chromatography (HPLC) using a previously described modular system (Anderson et al., 1988). The HPLC analyses were performed using a reverse-phase C_{18} (Altex Ultrasphere ODS, 4.6 mm × 15 cm, 5 μm) column with a mobile phase consisting of 4% acetonitrile in a phosphate buffer (pH 7.8, 0.01 M) for adenine elution and 10% acetonitrile in the same buffer for DDA elution.

Kinetic studies

Accurately weighed amounts of HP-β-CD (0–308 mg) were added to 2-ml volumetric tubes along with 40 μl of a fresh DDA stock solution (0.05 M). The samples were then diluted with an appropriate buffer ($\mu = 0.01$ (Perrin and Dempsey, 1979)), mixed, quickly transferred to septum sealed screw-top vials and placed in a circulating water bath (Haake model A81) at a controlled temperature (±0.1°C). At various intervals ranging from minutes to hours depending on the predicted hydrolysis rate, 200-μl samples were withdrawn and quickly diluted with 1 ml of pH 7.9 phosphate buffer (0.05 M) to stop further reaction. The diluted samples were then analyzed

by HPLC using external standards of DDA or adenine to determine sample concentrations. Sample pH values were measured at the reaction temperature using a standard pH meter (PH M 82, Radiometer America, Cleveland, OH) equipped with a combination pH electrode (81-03, Orion Research, Boston, MA). First-order rate constants were obtained from semilogarithmic plots of the disappearance of DDA vs time or from plots of adenine formation vs time (previous studies have shown that adenine is the only product forming under these conditions (Anderson et al., 1988)). A similar experiment was also conducted in 0.2 M D-glucose in place of HP-β-CD.

UV difference spectra

Accurately weighed amounts of HP-β-CD (0–770 mg) were added to 5-ml volumetric flasks and diluted with pH 8.95 ($\mu = 0.01$) phosphate buffer. Into a 2 ml volumetric (solution A) was added 100 μl of pH 8.95 buffer which was then diluted with one of the above HP-β-CD solutions. To another 2 ml volumetric (solution B) 100 μl of a DDA stock solution (4×10^{-3} M) in pH 8.95 buffer was added and diluted with the same HP-β-CD solution. Solution C was prepared by adding 100 μl of the DDA stock solution to a 2 ml volumetric and diluting with pH 8.95 buffer. This process was repeated for each of the HP-β-CD concentrations. The final DDA concentration was 2.0×10^{-4} M and ionic strength was $\mu = 0.01$ for all samples.

UV difference spectra were generated at various temperatures (± 0.1° C) using a UV-Vis double-beam spectrophotometer equipped with water-jacketed cell holders (Perkin-Elmer model Lambda-7) by the following procedure. Solution A was placed in the sample cell compartment with a pH 8.95 buffer solution in the reference cell compartment, and an electronic background correction was performed between 240 and 325 nm to subtract the HP-β-CD spectra from subsequent spectra. Solution B was then placed in the sample cell compartment while the reference cell compartment contained solution C. A scan from 240 to 325 nm produced a difference spectrum between free and complexed DDA.

pK_a determinations

DDA solutions were carefully prepared using deionized water which had been degassed and stored under nitrogen. HP-β-CD (0–308 mg) was carefully weighed and placed into a 2 ml volumetric. An accurately measured volume of an aqueous solution of DDA was then added to the 2 ml volumetric and diluted with degassed, deionized water, to give a final DDA concentration of 0.02 M. pK_a determinations were carried out under nitrogen at constant temperature (± 0.1° C) by adding an aliquot of a 1.00 M HCl solution equal to the amount necessary for half-neutralization to the solution using a calibrated digital pipet (Gilson, France) and measuring the pH. Using this method the pK_a of DDA could be determined quickly, before substantial degradation occurred (maximum degradation using this procedure was $< 1\%$). A validation of the accuracy of the pK_a values determined using this method was performed at 25° C using the complete titration curves, with ionic strength maintained constant ($\mu = 0.01$) with KCl. The two methods gave identical pK_a values to within 0.02 pH units.

Results

Inhibition of acid-catalyzed hydrolysis

First-order rate constants for the acid-catalyzed hydrolysis of DDA, k_{obs}, obtained from first-order plots of DDA disappearance vs time or from initial rates of adenine formation at various temperatures, pH values and HP-β-CD concentrations are reported in Table 1. As shown in Table 1, DDA hydrolysis is dramatically inhibited upon the addition of HP-β-CD. A similar hydrolysis experiment conducted in 0.2 M D-glucose at 25° C and pH 5.0 yielded a first-order rate constant of 2.1×10^{-3} h^{-1}, which agrees closely with the data obtained in buffer alone after correction for slight pH differences, indicating that 0.2 M D-glucose has no significant effect on the hydrolysis rate. This suggests that the inhibition of hydrolysis by HP-β-CD is not due to a solvent effect. At a given pH and temperature, the observed first-order rate constants decrease with increasing concentrations of HP-β-CD, illustrated graphically in Fig. 2 as a

TABLE 1

First-order hydrolysis rate constants for DDA at various settings of [HP-β-CD], pH, and temperature

Temperature (°C)	pH	k_{obs} (h^{-1})	[HP-β-CD]	Temperature (°C)	pH	k_{obs} (h^{-1})	[HP-β-CD]
4	2.09	0.0470	0.000	37	2.11	4.62	0.000
	2.10	0.0435	9.60E − 3		2.10	4.39	0.010
	2.08	0.0348	0.020		2.10	3.91	0.020
	2.09	0.0253	0.040		2.11	3.22	0.040
	2.10	0.0218	0.052		2.12	2.90	0.050
	2.10	0.0239	0.061		2.12	2.57	0.060
	2.10	0.0194	0.082		2.12	2.27	0.080
	2.10	0.0176	0.100		2.16	1.93	0.100
4	3.75	7.08E − 4	0.000	37	5.64	2.27E − 3	0.000
	3.78	4.95E − 4	0.011		5.68	1.49E − 3	0.010
	3.79	4.03E − 4	0.021		5.68	1.11E − 3	0.020
	3.89	2.22E − 4	0.043		5.72	6.21E − 4	0.050
	3.90	1.76E − 4	0.053		5.73	4.99E − 4	0.060
	3.91	1.46E − 4	0.064		5.76	3.51E − 4	0.100
	3.94	1.05E − 4	0.085				
	3.95	8.21E − 5	0.106	50	2.11	17.2	0.000
					2.11	15.1	0.010
4	4.22	3.75E − 4	0.000		2.12	12.5	0.020
	4.24	3.15E − 4	0.010		2.12	11.3	0.039
	4.26	2.16E − 4	0.020		2.11	11.1	0.050
	4.32	1.41E − 4	0.040		2.14	9.47	0.060
	4.32	1.26E − 4	0.050		2.15	8.53	0.080
	4.38	1.11E − 4	0.060		2.15	8.16	0.100
	4.42	7.52E − 5	0.080				
	4.47	5.71E − 5	0.100	50	3.75	0.600	0.000
					3.77	0.514	0.010
4	5.18	5.33E − 5	0.000		3.77	0.413	0.020
	5.19	3.76E − 5	9.50E − 3		3.83	0.278	0.040
	5.21	2.29E − 5	0.019		3.86	0.243	0.050
	5.24	1.40E − 5	0.040		3.87	0.213	0.060
	5.26	1.32E − 5	0.050		3.90	0.155	0.080
	5.27	1.11E − 5	0.060		3.92	0.127	0.100
	5.44	6.29E − 6	0.100				
				50	4.05	0.296	0.000
25	2.10	1.07	0.000		4.12	0.244	0.010
	2.10	0.945	0.010		4.16	0.192	0.020
	2.11	0.859	0.020		4.19	0.129	0.040
	2.14	0.689	0.040		4.21	0.114	0.050
	2.13	0.621	0.050		4.23	0.093	0.060
	2.13	0.569	0.060		4.26	0.079	0.080
	2.15	0.493	0.080		4.30	0.062	0.100
	2.19	0.397	0.100				
				50	5.71	7.70E − 3	0.000
25	5.17	1.25E − 3	0.000		5.75	5.40E − 3	0.010
	5.17	9.49E − 4	0.010		5.77	4.29E − 3	0.020
	5.20	6.41E − 4	0.020		5.76	3.16E − 3	0.040
	5.23	3.80E − 4	0.040		5.75	2.80E − 3	0.050
	5.26	3.31E − 4	0.050		5.79	2.23E − 3	0.060
	5.25	2.79E − 4	0.060		5.79	1.85E − 3	0.080
	5.26	2.31E − 4	0.080		5.81	1.68E − 3	0.100
	5.25	1.78E − 4	0.101				

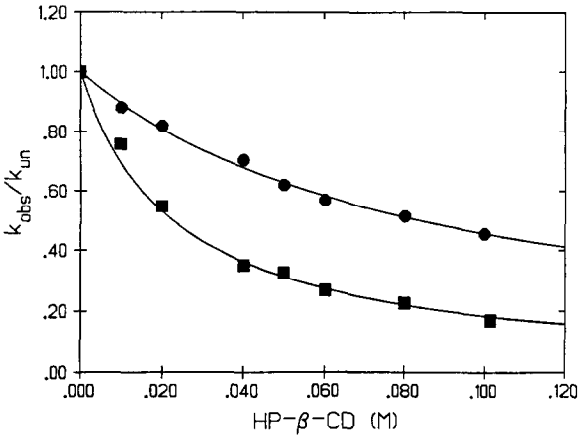
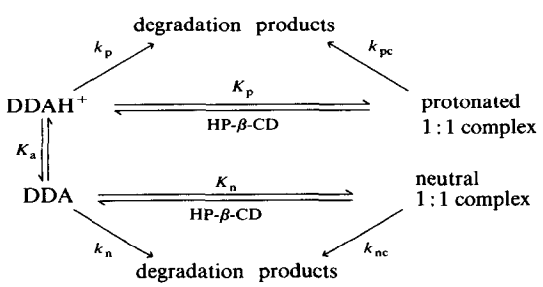


Fig. 2. Plot of k_{obs}/k_{un} vs [HP-β-CD] at 25 °C. (●) pH 2, (■) pH 5.

plot of the ratios of k_{obs}/k_{un} vs HP-β-CD concentration, at 25 °C and both pH 2 and 5, where k_{un} represents the observed first-order rate constant at the same pH in the absence of HP-β-CD. Assuming the pK_a of the conjugate acid form of DDA to be approx. 3.8 (see later results), approx. 94% of DDA would be estimated to be unprotonated at pH 5 while at pH 2, the proportion protonated is > 98%. Since reaction rates decrease significantly with added HP-β-CD at both pH 2 and 5, both the protonated and unprotonated species may form complexes. To model the kinetic results under these experimental conditions, the following reaction scheme (Scheme 1) was postulated, where K_p and K_n are the formation constants for the 1 : 1 complexes of the protonated and neutral species, k_p and k_n are the bimolecular rate constants for acid-catalyzed hydrolysis of uncomplexed $DDAH^+$ and neutral DDA, and k_{pc}



Scheme 1.

and k_{nc} are the bimolecular rate constants of acid-catalyzed hydrolysis of the corresponding complexed species.

Based on the above mechanism and under the experimental conditions employed the following rate equation can be derived:

$$k_{obs} = \{k_n K_a + k_p[H^+] + k_{nc} K_n[\text{HP-}\beta\text{-CD}] K_a + k_{pc} K_p[\text{HP-}\beta\text{-CD}][H^+]\} \times \{([H^+] + K_a)\}^{-1} f[H^+] \quad (1)$$

where

$$f = 1\{1 + [(K_n K_a[\text{HP-}\beta\text{-CD}]_t) + (K_p[H^+][\text{HP-}\beta\text{-CD}]_t)] \times [([H^+] + K_a)]^{-1}\}^{-1}$$

where f represents the fraction of DDA in the uncomplexed form. Because Eqn. 1 contains 7 unknowns, reliable estimates of the values of these parameters were not possible from the kinetic data alone. Therefore, independent experiments were conducted to obtain values for K_a, K_n, and K_p as a function of temperature.

Determination of K_n from UV difference spectra

An examination of the UV spectra of DDA in the presence and absence of HP-β-CD indicated that the absorption curve of DDA is shifted to higher wavelengths upon complex formation. UV difference spectra were therefore utilized to obtain independent estimates of K_n, the binding constant for 1 : 1 complex formation between HP-β-CD and the neutral form of DDA, as a function of temperature. Due to the instability of DDA in acidic solutions, this method could not be used for determining K_p, the complexation constant for the protonated form of DDA.

Typical UV difference spectra at 37 °C and pH 8.95, and at various HP-β-CD concentrations are shown in Fig. 3. The existence of an isosbestic point at 270 nm in Fig. 3 suggests the existence of

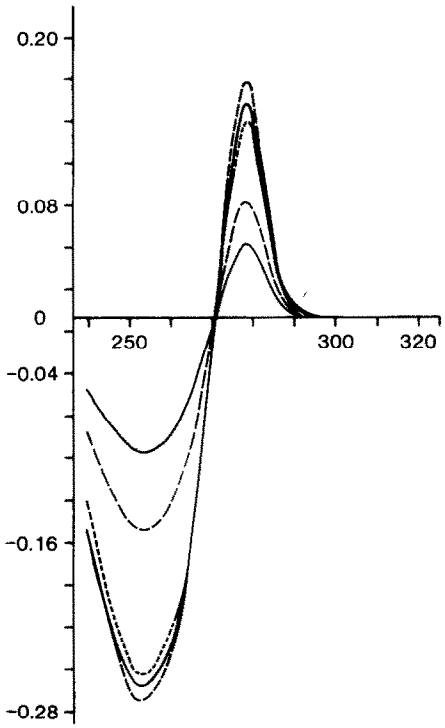


Fig. 3. UV difference spectra at [DDA] $2.0E-4$ M and 37 °C. [HP-β-CD] ranged from 0.01 to 0.1 M.

only 2 UV absorbing species in these solutions, supporting the assumption that only 1 : 1 complexes form between DDA and HP-β-CD.

Based upon the equilibria depicted in Scheme 1 which assumes that DDA forms only 1 : 1 complexes with HP-β-CD, the following equation can be derived for the peak-to-trough absorbance differences observed as the concentration of HP-β-CD is varied.

$$(\mathrm{Abs}_{max} - \mathrm{Abs}_{min}) = (\Delta\epsilon) K_n [\text{HP-}\beta\text{-CD}][\mathrm{DDA}] / (1 + K_n[\text{HP-}\beta\text{-CD}]) \quad (2)$$

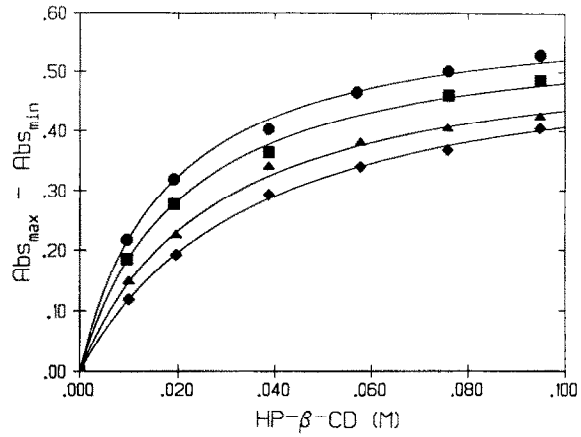


Fig. 4. Plot of $\mathrm{Abs}_{max} - \mathrm{Abs}_{min}$ vs [HP-β-CD] from UV spectra. (●) 4 °C, (■) 25 °C, (▲) 37 °C, (◆) 50 °C.

Fig. 4 illustrates the relationship between these absorbance differences and [HP-β-CD] at 4, 25, 37, and 50 °C. The curves represent Eqn. 2 with the values for K_n and $\Delta\epsilon$ optimized via non-linear least-squares regression analysis (MINSQ, MicroMath Scientific, Salt Lake City, UT). The K_n values obtained from these analyses are listed in Table 2.

Determination of K_a and K_p from pH titration data

Decreases in the apparent pK_a of the conjugated acid of DDA, pKa_{app}, were observed with increasing concentrations of HP-β-CD by titrating solutions of DDA varying in HP-β-CD concentration with a standardized solution containing 1.00 M HCl. Due to the instability of DDA in acidic media, a one-point titration procedure which could be completed in approx. 1 min was generally employed.

Decreases in pKa_{app} (or increases in Ka_{app}) were attributed to differences in the binding affin-

TABLE 2

Thermodynamic parameters for complex formation between 2',3'-dideoxyadenosine (DDA) with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and for the ionization of DDA in water (numbers in parentheses are standard deviations)

Parameter	4 °C	25 °C	37 °C	50 °C	$\Delta H°$ (kcal/mol)	$\Delta S°$ (e.u.)
K_n (M^{-1})	56.4 (2.7)	47.6 (2.6)	36.4 (2.0)	27.6 (1.6)	−2.8 (0.5)	−2.0 (2.1)
K_p (M^{-1})	18.1 (1.2)	12.3 (0.4)	10.4 (0.7)	9.4 (0.6)	−2.5 (2.5)	−3.5 (1.2)
K_a ($M \times 10^{-4}$)	1.10 (0.03)	1.47 (0.04)	1.91 (0.10)	2.47 (0.03)	3.1 (0.3)	7.0 (1.4)

ities of the neutral and protonated forms of DDA for HP-β-CD. Ka_{app} can be shown to vary with the total concentration of HP-β-CD ($[HP\text{-}\beta\text{-}CD]_t$), as shown in Eqns. 3 and 4, where K_a is the ionization constant for the conjugate acid of DDA in water and K_n and K_p were defined previously as the formation constants for the neutral and protonated complexes, respectively.

$$Ka_{app} = \frac{K_a(K_n[HP\text{-}\beta\text{-}CD]_f + 1)}{(K_p[HP\text{-}\beta\text{-}CD]_f + 1)} \quad (3)$$

and when $pH = pKa_{app}$,

$$[HP\text{-}\beta\text{-}CD]_t = [HP\text{-}\beta\text{-}CD]_f\left(1 + \frac{K_n[DDA]_t}{2(1 + K_n[HP\text{-}\beta\text{-}CD]_f)} + \frac{K_p[DDA]_t}{(1 + K_p[HP\text{-}\beta\text{-}CD]_f)^2}\right) \quad (4)$$

As shown by Eqns. 3 and 4, Ka_{app} and $[HP\text{-}\beta\text{-}CD]_t$, the dependent and independent variables in these experiments, are related through the implicit variable $[HP\text{-}\beta\text{-}CD]_f$, the concentration of uncomplexed HP-β-CD.

Apparent pK_a values at various temperatures and HP-β-CD concentrations are reported in Table 3 and plotted as Ka_{app} vs [HP-β-CD] in Fig. 5. The curves were calculated using Eqns. 3 and 4 and a non-linear least-squares regression analysis to obtain best estimates for K_p with K_a obtained from titration in the absence of HP-β-CD and K_n determined from the UV difference spectra. When both K_n and K_p were treated as unknown parameters, the values obtained for K_n were not significantly different from K_n values determined using the UV difference spectra. Table 2 lists the values of K_a and the complex formation constants for both the neutral and protonated species, K_n, and K_p, obtained from the difference spectra and the data in Table 3, respectively.

Using the relation of van't Hoff, Eqn. 5, enthalpies and entropies of complex formation and the enthalpy and entropy of ionization of DDA were calculated by a least-squares regression analysis. These values are listed in Table 2.

$$\ln(K_f) = \Delta S^\circ/R - \Delta H^\circ/RT \quad (5)$$

TABLE 3

Experimental pKa_{app} values of DDA in solutions containing various HP-β-CD concentrations ($\mu = 0.01$)

Temperatures (°C)	[HP-β-CD]	pKa_{app}
4	0.00	3.95
	0.00	3.95
	0.00	3.97
	0.02	3.86
	0.02	3.82
	0.04	3.77
	0.05	3.72
	0.08	3.65
	0.10	3.61
	0.10	3.53
	0.10	3.53
25	0.00	3.82
	0.00	3.84 [a]
	0.00	3.84 [a]
	0.05	3.54
	0.10	3.44
37	0.00	3.69
	0.00	3.75
	0.05	3.48
	0.05	3.45
	0.10	3.40
	0.10	3.37
50	0.00	3.61
	0.00	3.60
	0.00	3.61
	0.05	3.41
	0.10	3.36
	0.10	3.32
	0.10	3.36
	0.10	3.28

[a] pKa_{app} determined from complete titration curve ($\mu = 0.01$).

Determination of bimolecular rate constants for the acid-catalyzed hydrolysis of free and complexed DDA species

With reliable estimates of the pK_a for the conjugate acid form of DDA and complex formation constants for the protonated and neutral species as a function of temperature, obtained from the

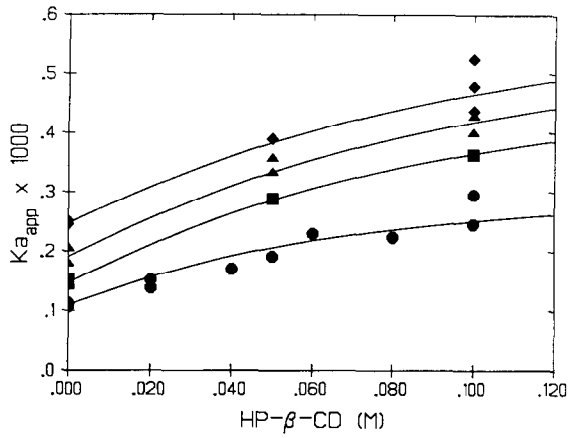


Fig. 5. Plot of Ka_{app} vs [HP-β-CD]. (●) 4 °C, (■) 25 °C, (▲) 37 °C, (◆) 50 °C.

UV difference spectra and apparent pK_a determinations, it is now possible to apply Eqn. 1 to the data for the observed first-order hydrolysis rates of DDA vs [HP-β-CD]$_t$ to obtain values for the bimolecular rate constants k_n, k_p, k_{nc}, and k_{pc}, defined in Scheme 1. The relevant parameter values were obtained from non-linear regression analyses of the kinetic data, treating K_u, K_p, and K_a as constants during fitting. Bimolecular rate constants for the protonated and neutral complexes at various temperatures are reported in Table 4. The values of k_{nc} and k_{pc} were not significantly different from zero (standard deviations larger than the parameter estimates).

Activation enthalpies and entropies for the bimolecular rate constants from Eqn. 1 were calculated using the Eyring equation (Eqn. 6) and are also listed in Table 4.

$$k_i = (kT/h)\left(e^{\Delta S^{\ddagger}/R}\right)e^{-\Delta H^{\ddagger}/RT} \qquad (6)$$

Discussion

Inhibition of DDA hydrolysis by cyclodextrin complexation

An important finding of this study is that the acid-catalyzed hydrolysis of DDA is completely inhibited in HP-β-CD complexes. However, due to the small binding constants of both the protonated and neutral complexes the maximum stabilization attainable in a 0.1 M HP-β-CD solution at 25 °C is 5-fold at pH 5 and only 2-fold at pH 2. Cyclodextrin complexation is therefore not highly effective in stabilizing DDA in an acidic environment. Nevertheless, the approach may have utility in stabilizing other dideoxynucleosides if their binding affinities are significantly larger.

The observation that the reactivity of DDA in HP-β-CD complexes is completely inhibited may be rationalized by considering the reaction mechanism and the structure of the inclusion complex. Previous studies (Garrett and Mehta, 1972; Romero et al., 1978; York, 1981) suggest that the transition state for the acid-catalyzed hydrolysis of purine nucleosides has a considerable amount of oxocarbonium ion character (Fig. 6). Evidence favoring this transition state includes the following: (1) entropies of activation for these reactions are usually near zero or positive (Garrett and Mehta, 1972; York, 1981; Anderson et al., 1988); (2) the rates of reaction are highly sensitive to the hydroxyl substituents in the sugar ring (Garrett and Mehta, 1972; York, 1981; Anderson et al., 1988); and (3) rather large secondary α deuterium isotope effects are observed (Romero et al., 1978). This oxocarbonium ion transition state would be expected to be destabilized in the more hydro-

TABLE 4

Bimolecular rate constants and thermodynamic parameters for the hydrolysis of 2',3'-dideoxyadenosine (DDA) (numbers in parentheses are standard deviations)

Parameter	4 °C	25 °C	37 °C	50 °C	Activation parameters	
					$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (e.u.)
k_n (1 mol^{-1} h^{-1})	6.8 (1.2)	193 (5)	927 (29)	4099 (122)	24.7 (0.4)	34.6 (1.5)
k_p (1 mol^{-1} h^{-1})	4.4 (1.0)	135 (3)	580 (18)	2064 (96)	24.4 (0.9)	32.5 (3.3)
k_{nc} (1 mol^{-1} h^{-1})	5e − 3 (1)	0.25 (1.72)	1.0 (15)	1e − 7 (79)	- - - - - -	- - - - - -
k_{pc} (1 mol^{-1} h^{-1})	3e − 3 (1)	4.8 (4.9)	0.2 (30.0)	94 (190)	- - - - - -	- - - - - -

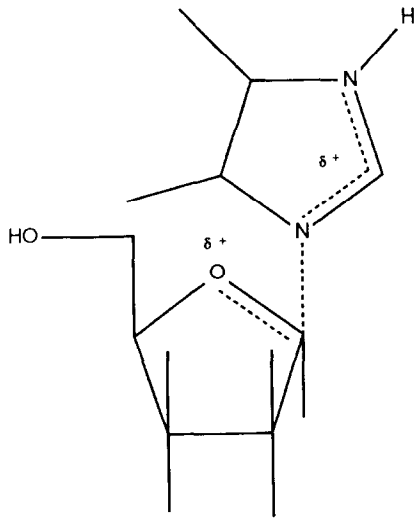


Fig. 6. Oxycarbonium ion transition state.

phobic environment of the cyclodextrin cavity.

Recent evidence (Xiang and Anderson, 1989) generated in these laboratories suggests that probable orientations for the neutral and protonated forms of adenine nucleosides in the cyclodextrin cavity are as shown in Fig. 7A and B, respectively. In the neutral complexes, the purine residue is believed to be oriented in the complex with its short axis nearly parallel to the C_7 axis of the β-CD cavity. In this orientation, it does not appear that the ribosyl moiety, the site of oxocarbonium ion formation, would lie buried in the cyclodextrin cavity. However, a reduction in reactivity may also result from the effective block of protonation at the N-7 position of the adenine ring upon complex formation. Protonation at N-7, while less favorable than at the N-1 position (Christensen et al., 1970) is believed to occur along the reaction pathway for C–N bond hydrolysis (Alivisatos et al., 1962; Holmes and Robins, 1965). Principal evidence for this comes from studies of the hydrolysis of 8-substituted adenosines (Alivisatos, 1962). The hydrolysis of 8-aminoadenosine in 1 M HCl was complete in less than 15 min, whereas 8-hydroxyadenosine was essentially unchanged after 2 h under the same conditions. Since 8-hydroxyadenosine probably exists in the keto form, protonation of the imidazole ring would be difficult. In an inclusion complex, a transition state in which the purine residue is protonated at N-7 would be destabilized, as this site is well within the complex for both the protonated and neutral species as depicted in Fig. 7.

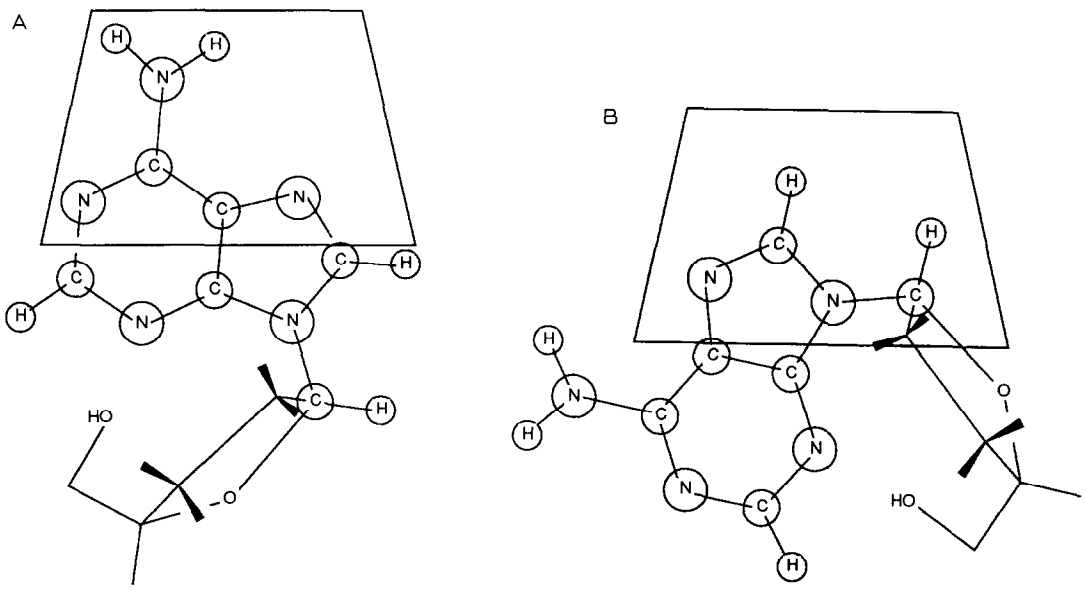


Fig. 7. Possible inclusion geometries of DDA with HP-β-CD. (A) Neutral complex principal geometry. (B) Protonated complex geometry.

Temperature effects

Whereas enthalpies of cyclodextrin complex formation are typically negative, entropies may be positive or negative, depending on the relative contributions of various forces such as non-specific van der Waals forces, hydrogen bonding, and hydrophobic interactions and the allowed orientations of the guest within the cyclodextrin cavity (Lewis and Hansen, 1973; Tabushi et al., 1978). Consistent with previous work, the enthalpies for complex formation between DDA and HP-β-CD are negative, indicating weaker binding with increasing temperature. The lower values of ΔG_f° for the protonated complex appear to be due largely to a more negative entropy of formation, although the uncertainties in these parameters do not allow one to draw firm conclusions.

Further information on the orientation of purine nucleosides within the cyclodextrin cavity can be obtained by exploring structure-binding relationships as the structure of the guest and cyclodextrin cavity size are varied and through the use of various spectroscopic techniques. A subsequent report will discuss the results of these studies.

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References

Alivisatos, S.G.A., Lamantia, L. and Matijevich, B.L., Imidazolytic processes. 6. Enzymic formation of benzimidazole and 5,6-dimethyl-benzimidazole containing dinucleotides. *Biochim. Biophys. Acta*, 58 (1962) 209–217.

Anderson, B.D., Wygant, M.B., Xiang, T.-X., Waugh, W.A. and Stella, V.J., Preformulation solubility and kinetic studies of 2′,3′-dideoxypurine nucleosides: Potential anti-AIDS agents. *Int. J. Pharm.*, 45 (1988) 27–37.

Bender, M.L. and Komiyama, M., *Cyclodextrin Chemistry*, Springer, Berlin, 1978, pp. 1–79.

Christensen, J.J., Rytting, J.H. and Izatt, R.M., Thermodynamic pk, ΔH°, ΔS°, and ΔC_p° values for proton dissociation from several purines and their nucleosides in aqueous solution. *Biochemistry*, 9 (1970) 4907–4913.

DeVita, V.T., Broder, S., Fauci, A.S., Kovacs, J.A. and Chabner, B.A., Developmental therapeutics and the acquired immunodeficiency syndrome. *Ann. Intern. Med.*, 106 (1987) 568–581.

Garrett, E.R. and Mehta, P.J., Solvolysis of adenine nucleosides 1. Effects of sugars and adenine substituents on acid-catalyzed hydrolysis. *J. Am. Chem. Soc.*, 94 (1972) 8532–8542.

Griffiths, D.W. and Bender, M.L., Cycloamyloses as catalysts. *Adv. Catal.*, 23 (1973) 209–261.

Holmes, R.E. and Robins, R.K., Purine nucleosides. 9. Synthesis of 9-beta-D-ribofuranosyl uric acid and other related 8-substituted purine ribonucleosides. *J. Am. Chem. Soc.*, 87 (1965) 1772–1776.

Jones, S.P., Grant, D.J.W., Hadgraft, J. and Parr, G., Cyclodextrins in the pharmaceutical sciences. *Acta Pharm. Technol.*, 30 (1984) 213–277.

Lewis, E.A. and Hansen, L.D., Thermodynamics of binding of guest molecules to α- and β-cyclodextrins. *J. Chem. Soc. Perkin II* (1973) 2081–2085.

Perrin, D.D. and Dempsey, B., *Buffers for pH and Metal Control*, Chapman and Hall, London, 1979, p. 45.

Romero, R., Stein, R., Bull, H.G. and Cordes, E.H., Secondary deuterium-isotope effects for acid-catalyzed hydrolysis of inosine and adenosine. *J. Am. Chem. Soc.*, 100 (1978) 7620–7624.

Szejtli, J., *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982, pp. 95–101.

Tabushi, I., Cyclodextrin catalysis as a model for enzyme action. *Acc. Chem. Res.*, 15 (1982) 66–72.

Tabushi, I., Kiyosuke, Y., Sugimoto, T. and Yamamura, K., Approach to the aspects of driving force of inclusion by α-cyclodextrin. *J. Am. Chem. Soc.*, 100 (1978) 916–919.

Uekama, K., Hirayama, F., Yamada, Y., Inaba, K. and Ikeda, K., Improvements of dissolution characteristics and chemical stability of 16,16-dimethyl-trans-delta-2-prostaglandin-E1-methyl-ester by cyclodextrin complexation. *J. Pharm. Sci.*, 68 (1979) 1059–1060.

Vanetten, R.L., Sebastian, J.F., Clowes, G.A. and Bender, M.L., A pictorial description of the 'Lock and Key' theory. *J. Am. Chem. Soc.*, 88 (1966) 2318–2319.

Xiang, T-X. and Anderson, B.D., Inclusion complexes of purine nucleosides with cyclodextrins: II. Investigation of inclusion complex geometry and cavity microenvironment. *Int. J. Pharm.*, 59 (1990) 45–55.

York, J.L., Effect of the structure of the glycon on the acid-catalyzed hydrolysis of adenine nucleosides. *J. Org. Chem.*, 46 (1981) 2171–2173.

Yoshida, A., Arima, H., Uekama, K. and Pitha, Pharmaceutical evaluation of hydroxyalkyl ethers of β-cyclodextrin. *Int. J. Pharm.*, 46 (1988) 217–222.