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Reduction of the Syn–Anti Glycosyl Conformational Barrier in 2'-Deoxyadenosine upon Binding to Ethidium Bromide. Evidence from Ultrasonic Relaxation Measurements[1]

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Abstract: The unimolecular relaxation found by ultrasonic relaxation in dilute aqueous solutions of 2'-deoxyadenosine was examined in the absence and presence of ethidium bromide (a model for an intercalating drug) and indole-3-acetic acid at pH 7.0 (a model for tryptophan as a potential binding site on a protein). This relaxation, which had previously been assigned to the syn-anti glycosyl isomerization, changes both in terms of f_r and amplitude in the presence of the added reagents. Both ethidium bromide and indole-3-acetic acid shift the relaxation frequency, f_r, to higher values. Detailed analysis of the data in the presence of varying amounts of ethidium bromide indicates that the apparent activation energy to syn-anti isomerization is decreased when 2'-deoxyadenosine is bound to ethidium bromide. ^{1}H NMR studies were performed to elucidate the mechanism of binding. Assuming a 1:1 2'-deoxyadenosine-ethidium bromide (heterostack) complex, ^{1}H NMR (in ^2H_2O) gives a $K_{heterostack}$ of ca. 300 M^{-1} compared to the value derived from the ultrasonic data (in H_2O) of ca. 400 M^{-1}.

Introduction

For several years now this laboratory has been engaged in developing the ultrasonic relaxation approach to study the kinetics and thermodynamics of the syn-anti glycosyl isomerization in nucleosides and nucleotides.[3-5] Based on our results as well as on those of Rhodes and Schimmel[6] it has been well established that adenosine,[3,6] 2'-deoxyadenosine,[6] and adenosine 3',5'-cyclic monophosphate[4] give rise to a unimolecular relaxation in the 10-100-MHz frequency range. With nucleotides the relaxation is masked by other fast processes.[7] We have also demonstrated that urea destacks aggregated nucleic bases[8] and that the stacking and syn-anti glycosyl conformational degree of freedom are coupled to each other.[4] While the method appears to be applicable primarily to purine nucleosides (in our hands a variety of pyrimidine nucleosides did not give rise to unimolecular relaxation in the frequency range 10-300 MHz accessible on our instrumentation), there are several aspects of the behavior of these molecules that are worthy of delineation and delineation of which can uniquely be performed by our technique.

One of the important questions concerning the solution behavior of nucleosides and nucleotides has to do with possible changes in syn-anti rotational barrier and equilibrium constant upon binding either to a receptor or to an intercalating drug. We have now examined that ultrasonic relaxation of 2'-deoxyadenosine attributable to the syn-anti isomerization in the presence of indole-3-acetic acid (a model for tryptophan as potential hydrophobic binding site on a protein) and ethidium bromide (as a model for an intercalating drug). Our choice of 2'-deoxyadenosine is dictated by the fact that it has relatively high solubility compared to other naturally occurring purine nucleosides[9] and the amplitude of the ultrasonic relaxation is large enough for this molecule to be readily quantitated. Herein we report our results which for the first time demonstrate that binding of 2'-deoxyadenosine to ethidium bromide or indol-3-acetic acid reduces the barrier to syn-anti rotations, i.e., increases f_r for the process. In addition, to confirm the ultrasonic results, we have determined the $K_{association}$ between ethidium bromide and 2'-deoxyadenosine by ^{1}H NMR, to our knowledge the first determination of this association constant between this intercalator and a nucleoside. The technique we have employed should be of general applicability.

Experimental Section

Materials. 2'-Deoxyadenosine and ethidium bromide were from Sigma; indole-3-acetic acid was from Eastman. All of these chemicals were used without further purification. Distilled, deionized, degassed water was employed for all solutions. pH was adjusted and measured with a Radiometer pH meter No. 26 equipped with glass and calomel electrodes.

Methods. Ultrasonic relaxation measurements were taken either on a pulse or a swept frequency resonator instrument. The measurements of the ultrasonic absorption were carried out at the odd harmonic frequencies of a 5-MHz X-cut quartz transducer by means of the pulse technique (MATEC Model 765 and 960). The frequency range was from 15 to 280 MHz.[10] A swept frequency resonator was also used for the absorption measurements in the frequency range of 12.5-38 MHz (the instrumentation employed is similar to Eggers' design[11]). The sound velocity was measured at 15 MHz. The ultrasonic cell was immersed in a water bath which was maintained within ±0.02°C.

^{1}H NMR measurements were performed on a JEOL PSFT-100 Fourier transform instrument at 28 °C (probe temperature). Chemical shifts were measured against 3-trimethylsilyl-*2,2,3,3-*$^2H_4$-propionic acid sodium salt (Stohler Isotope) in 99.8% ^2H_2O (Stohler Isotope). Typically 50-1000 transients of ca. 4 s repetition time and 45-60° pulse width were collected. For the most dilute samples the WEFT[12] technique was employed attempting to beat down the residual HO^2H signal by collecting transients with the $(180\text{-}\tau\text{-}90)T_1$ sequence where τ is the delay appropriate to null the HO^2H signal. On account of the very limited solubility of both ethidium bromide and 2'-deoxyadenosine, but the enhanced solubility of each in the presence of the other, several methods were attempted to prepare the samples. The method finally adopted involved preparing a stock solution of ethidium bromide (0.0025 M) and directly weighing in the 2'-deoxyadenosine into 5-mL volumes of this solution.

0002-7863/80/1502-3913$01.00/0

Table I. Results of Ultrasonic Absorption Measurements at 20 °C

2′-deoxyadenosine, M	indole-3-acetic acid, M	δ[a]	$10^{17}A$,[b] $s^2\ cm^{-1}$	$10^{17}B$,[b] $s^2\ cm^{-1}$	f_r, MHz	$10^{-5}c$,[c] $cm\ s^{-1}$
0.060	0.001		7.4	25.8	25	1.50
0.060	0.025 ethidium bromide		~3	26.0	~65	1.49
0.040	0		7.5	25.0	17	1.48
0.060	0	0	15.7	25.3	17	1.49
0.060	0.005	0.176	10.8	25.8	25	1.49
0.060	0.010	0.342	13.0	25.0	30	1.51
0.060	0.025	0.725	12.8	25.7	33	1.50
0.060	0.060	0.936	18.5	25.2	35	1.50
0.050	0.050	0.920	12.0	26.2	35	1.50
0.070	0.035	0.793	14.1	26.8	30	1.50
0.020	0.060	~1		~29.0		
0.060 (in 7 M urea)	0	0	14.6	21.4	15	1.68

[a] δ is from eq 3 in text. [b] A is the maximum amplitude, B is the base line, and f_r is the relaxation frequency. [c] The sound velocity.

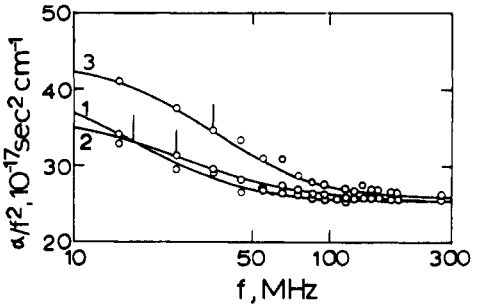


Figure 1. $10^{17}\alpha/f^2$ vs. f plots for (1) 0.06 M 2′-deoxyadenosine; (2) 0.06 M 2′-deoxyadenosine + 0.005 M ethidium bromide; (3) 0.06 M 2′-deoxyadenosine + 0.06 M ethidium bromide.

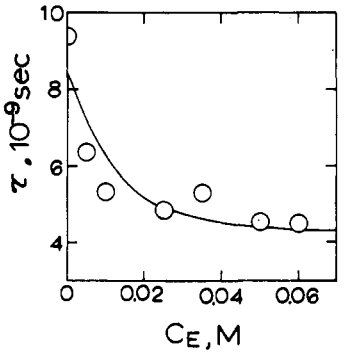


Figure 2. The dependence of the relaxation time, τ, on the concentration of ethidium bromide.

Results and Discussion

Figure 1 illustrates some typical ultrasonic absorption spectra obtained in aqueous solutions of 2′-deoxyadenosine in the absence and presence of varying amounts of ethidium bromide. All of the spectra measured are characterized by a single relaxation process and can be analyzed according to the equation

$$\alpha/f^2 = A/(1 + (f/f_r)^2) + B \qquad (1)$$

where α is the absorption coefficient, f_r is the relaxation frequency, and A and B are constants corresponding to the maximum amplitude and the base line, respectively. The relaxation frequency observed by us for 2′-deoxyadenosine (Table I) is considerably lower than that reported by Rhodes and Schimmel[6] (80 MHz at 24 °C). Within experimental error the relaxation frequency of 2′-deoxyadenosine was found to be independent of the concentration of nucleoside and based on results from Rhodes and Schimmel[6] and from our laboratory[3,4] the unimolecular relaxation can be associated with the syn–anti glycosyl isomerization.

The addition of indole-3-acetic acid alters the amplitude of the excess absorption and shifts the relaxation frequency to higher values (Table I). This implies that the rotation around the glycosyl bond is facilitated since the relaxation frequency f_r is expressed as

$$1/\tau = 2\pi f_r = k_f + k_r \qquad (2)$$

for an isomerization process where k_f and k_r are the unimolecular forward and reverse rate constants, respectively. As the amplitude of excess absorption is rather small in this case, a quantitative analysis of the ultrasonic properties is not warranted.

The addition of ethidium bromide, however, brings about larger changes that can be investigated in more detail. The solubility of ethidium bromide in H_2O is very limited (less than 0.025 M). The solubility of this compound dramatically increases in the presence of 2′-deoxyadenosine. Ethidium bromide is known to undergo self-association in aqueous solution;[13] it was therefore important to demonstrate that the observed relaxation was not due to this process. In the presence of 0.02 M 2′-deoxyadenosine a 0.06 M solution of ethidium bromide did not give rise to a chemical relaxation. This condition was selected since the presence of the nucleoside increases the solubility of the dye; on the other hand, 0.02 M 2′-deoxyadenosine itself gives rise to a chemical relaxation of negligible amplitude (on our instrumentation). Figure 2 and Table I list the results obtained by a series of additions of ethidium bromide to 2′-deoxyadenosine. We assume that the ultrasonic spectrum consists of two overlapping relaxation processes with similar relaxation times and amplitudes. What is measured therefore is a concentration-weighted average of the relaxation times. The relaxation frequency changes continuously with increased concentration of ethidium bromide. This strongly suggests that the cause of the excess absorption is that found for 2′-deoxyadenosine alone. The results also demonstrate that, although there is a shift in relaxation frequency, the relaxation observed is still that due to the syn–anti isomerization and not to the kinetics of heterostack (i.e., ethidium bromide–2′-deoxyadenosine) formation. If additional excess absorption had existed in the solution with ethidium bromide, the amplitude of excess absorption should have increased with increasing concentration of ethidium bromide.

Next the temperature dependence of f_r was determined for 2′-deoxyadenosine (0.06 M) in the absence and presence of ethidium bromide (0.025 M). Figure 3 illustrates the results of such studies. The apparent activation energy is less in so-

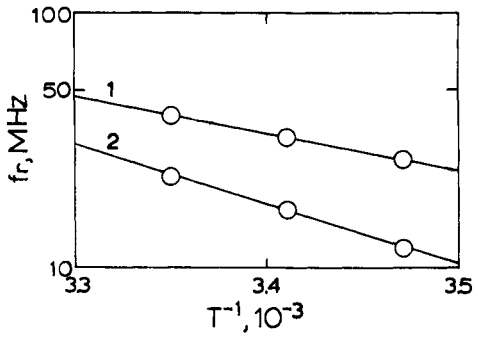


Figure 3. Determination of the apparent activation energy for glycosyl isomerization of 2'-deoxyadenosine with ethidium bromide ($E_{a\ app}$ = 6 kcal mol^{-1}; see line 1) and without intercalator ($E_{a\ app}$ = 11 kcal mol^{-1}; see line 2).

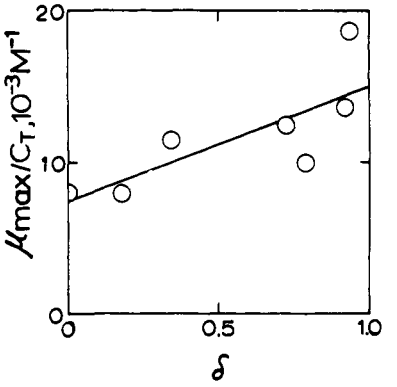


Figure 4. Plot of μ_{max}/C_T vs. δ as suggested by eq 7 in the text.

lutions with ethidium bromide (6 kcal/mol) compared to that without ethidium bromide (11 kcal/mol). This result is consistent with the finding that the f_r increases upon addition of ethidium bromide.

A model that accounts for the observed data is shown in Scheme I, where A and S are the anti and syn conformers and A' and S' are those when ethidium (E) bound, respectively. As the self-stacking constant for 2'-deoxyadenosine is known from the literature,[14,15] the monomer concentration can be estimated. This scheme predicts three relaxation times. We will assume that the normal mode most nearly approximated by the chemical reaction of binding ethidium bromide to the base is below the frequency range studied in this work. We base this assumption on the fact that the absorption of sound at high frequencies is similar to that of water and on literature results which for binding proflavin[16,17] or actinomycin[18] to polynucleotides have rate constants in the range 10^6-10^7 M^{-1} s^{-1} which, for our concentrations, would predict relaxation times in the kilohertz region. Of the two remaining relaxations we will assume that we observe both but that the relaxation frequencies of these processes are very similar and thus we observe an average relaxation time.[17] In fact since μ_{max} measures τ^{-1} directly what we observe is $\langle\tau^{-1}\rangle$. We can analyze the results using the techniques devised by Schwarz[19] for mean relaxation times. As a zeroth approximation we assume that both relaxation times are identical. Then we can write the maximum excess absorption per wavelength μ_{max} as follows:

$$\mu_{max} = (\pi/2\beta RT)(\Delta V_s)^2\Gamma^{-1} \quad (3)$$

$$\Delta V_s = \Delta V - (\theta/\rho C_p)\Delta H \text{ and } \Gamma^{-1} = K_u C_T/(1 + K_u)^2 \quad (4)$$

where ΔV is the standard volume change, ΔH is the enthalpy change, β is the adiabatic compressibility, θ is the thermal expansion coefficient, ρ is the density, C_p is the specific heat at a constant pressure, C_T is the total concentration which participates in the reaction, and K_{au} is the equilibrium constant of anti-syn forms. In the solution with ethidium bromide, eq 4 may be written as

$$\mu_{max} = \frac{\pi}{2\beta RT}[(\Delta V_{sa})^2\Gamma_a^{-1} + (\Delta V_{sa})^2\Gamma_u^{-1}]$$

$$= \frac{\pi}{2\beta RT}\left[(\Delta V_{sa})^2\frac{K_a}{(1+K_a)^2}\delta C_T + (\Delta V_{su})^2\frac{K_u}{(1+K_u)^2}(1-\delta)C_T\right] \quad (5)$$

where subscript "a" in ΔV_s and K refers to the equilibrium of II and "u" that of I in Scheme I and $\delta = (1/C_m)((A') + (S'))$. Therefore, the plots of μ_{max}/C_T vs. δ should be straight lines. These are shown in Figure 4. This plot gives

$$\frac{(\Delta V_{su})^2}{C_T}\frac{K_u}{(1+K_u)^2} = 8\times10^{-3} \text{ and } \frac{(\Delta V_{sa})^2}{C_T}\frac{K_a}{(1+K_a)^2} = 22\times10^{-3} \quad (6)$$

K_a is the syn-anti equilibrium constant for bound deoxyadenosine. Hence the magnitude of the ultrasonic effect in the bonded form is greater than that of the unbonded form. This is also evident from Figure 1. Unfortunately we cannot resolve this increase into either a change in K or a change in ΔV_s.

We can now use the relative magnitude to obtain a better approximation to τ^{-1} for the monomer with bound ethidium ion. According to Schwarz[19] for a distribution of j closely spaced relaxations the average reciprocal relaxation time $\langle\tau^{-1}\rangle$

$$\langle\tau^{-1}\rangle = \sum_j \frac{B_j}{\tau_j} \quad (7)$$

where B_j is a normalized amplitude for process j with relaxation time τ_j. From the previous amplitude calculation, we have

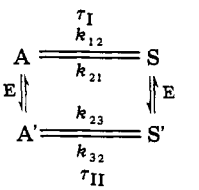
$$\langle\tau^{-1}\rangle = \frac{(8\times10^{-3})\tau_1^{-1}(1-\delta)}{(8\times10^{-3}) + (14\times10^{-3})\delta} + \frac{(22\times10^{-3})\tau_2^{-1}\delta}{(8\times10^{-3}) + (14\times10^{-3})\delta} \quad (8)$$

where $\delta = ([A'] + [S'])/C_m$. With the further definition of $K = [A']/[E][A] = [S']/[E][S]$ and C_E and C_m being the initial concentrations of ethidium bromide and monomer concentration of 2'-deoxyadenosine, subtracting the self-stacked contribution (i.e., C_m = free + ethidium bromide bound), respectively, one obtains

$$\delta = (1/2KC_m)[K(C_m + C_E) + 1 - ((K(C_m - C_E) + 1)^2 + 4KC_E)^{1/2}] \quad (9)$$

K can be obtained from the best fit of the experimental data to eq 3. The value so obtained is $K = 4\times10^2$ M^{-1} and $\tau_1 = 8.4\times10^{-9}$ s and $\tau_2 = 3.2\times10^{-9}$ s. Thus, when the ethidium ion binds, the syn-anti process is accelerated, being 2.6 times faster in the bound form than in the free form. To be consistent we should correct our amplitude calculation for the difference in τ. The change made by this correction is less than that caused by the errors in the binding constant and thus is not significant.

Scheme I



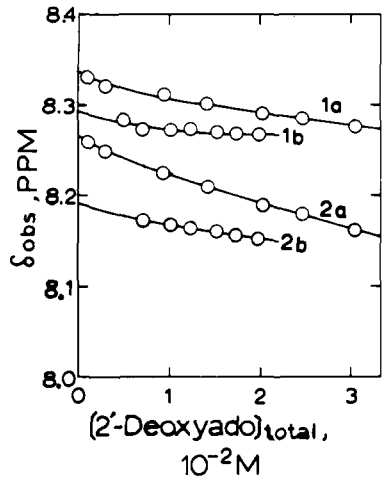


Figure 5. Concentration dependence of the chemical shifts of C8-H (curves 1a and 1b) and C2-H (curves 2a and 2b) in the absence (the a curves) and in the presence (the b curves) of 0.0025 M ethidium bromide.

To test if the K obtained in the ultrasonic studies is reasonable we have also performed high-resolution ^1H NMR studies on the binding mechanism of ethidium and 2′-deoxyadenosine. It would have been easiest to treat the data according to the NMR analogue of the Benesi-Hildebrand plot, with low constant concentration of 2′-deoxyadenosine and at least ten times more concentrated and variable concentrations of ethidium bromide. This protocol failed on account of the overlap of the two types of aromatic protons resonances. The protocol adopted involved first measuring the concentration dependence (down to 0.001 M) of the chemical shifts in 2′-deoxyadenosine alone, and next in the presence of 0.0025 M ethidium bromide. Such chemical shifts measured from sodium 3-trimethylsilyl-*2,2,3,3-*$^2H_4$-propanoate are indicated in Figure 5. The C2-H and C8-H of the adenine base are readily resolvable. The assignment is readily made since even in neutral solution the C8-H is well documented to exchange with ^2H much faster than does the C2-H. The downfield shift observed upon dilution for both base protons is consistent with the known self-stacking of 2′-deoxyadenosine and the diamagnetic ring current shifts accompanying such behavior. The well-behaved functionality of the δ_{obsd}-concentration plots assures reliable extrapolation to monomer chemical shifts (infinitely dilute). With the known $K_{self\text{-}stack}$ of 2′-deoxyadenosine of 13[14,15] and the usual assumption of fast exchange of the 2′-deoxyadenosine among the various environments, the following relationships obtain for the δ_{obsd}, the observed chemical shift of the base protons:

$$\delta_{obsd} = \delta_{free}\, n + \delta_{stack}\, m + \delta_{heterostack}\, q \qquad (10)$$

where δ_{free}, δ_{stack}, and $\delta_{heterostack}$ are the chemical shifts of monomeric nucleoside, self-stacked nucleoside, and ethidium bromide bound nucleoside, respectively, and n, m, q are the corresponding mole fractions of the protons in the three environments. δ_{free} can be read off the appropriate curve in Figure 5. Alternatively both δ_{free} and δ_{stack} can be determined from the relationship involving the concentration dependence of the C2-H and C8-H resonances in the absence of ethidium bromide and assuming 13 for the self-stacking constant[14,15] by plotting δ_{obsd} vs. mole fraction of free nucleoside. The values obtained (ppm) are 8.336 and 8.193 for C8-H and 8.269 and 8.072 for C2-H for δ_{free} and δ_{stack}, respectively. The fact that increased concentration of 2′-deoxyadenosine caused an upfield shift (even though less and less ethidium bromide is bound) clearly indicates that both self-stacking and heterostacking equilibria must be taken into account. To determine $K_{heterostack}$ we employed a derivation which to our knowledge was first employed by Dahlquist and Raftery, et al., in the determination of the $K_{dissociation}$ of lysozyme-inhibitor complex.[20]

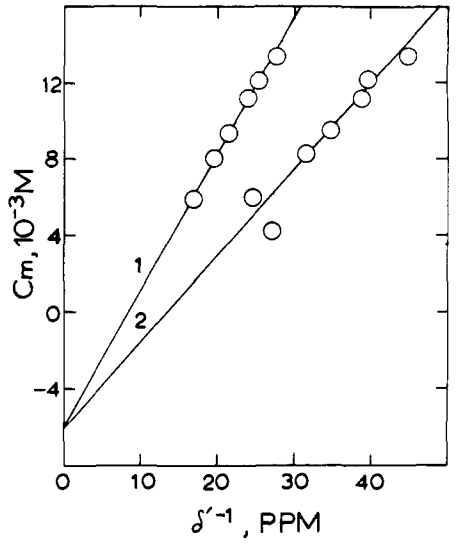


Figure 6. Analysis of the data presented in Figure 5 and as treated in eq 9 of the text. The lines represent the linear least-squares "best" value and are superimposed on the experimental data. Line 1 is for the C2-H data, line 2 for the C8-H data. The intercept on the ordinate is related to the K_{diss}; the slopes are related to the bound chemical shift in the ethidium bromide-2′-deoxyadenosine complex.

Assuming formation of a 1:1 ethidium bromide-2′deoxyadenosine complex the expression one can employ is

$$C_m = E_0\Delta/\delta' - K_{diss} - E_0 \qquad (11)$$

where δ' is a corrected δ_{obsd}, i.e.

$$\delta' = \delta_{free} - \delta_{obsd} - (\delta_{free} - \delta_{stack})n \qquad (12)$$

where the last terms on the right can be obtained from the self-stacking behavior in Figure 5 and Δ is the total chemical shift between infinitely dilute and ethidium bromide bound protons. Rigorously one has to cycle by an iterative procedure to estimate the mole fraction of stacked nucleoside from knowledge of the $K_{self\text{-}stack}$ and $K_{heterostack}$, then repeat calculation of $K_{heterostack}$. We have taken such calculation through one cycle and the correction was much less than 10%; i.e., such correction is not warranted in view of the error in the experimental determination.

The $K_{association}$ values so determined are 2.7×10^2 and 3.0×10^2 M^{-1} from data on C8-H and C2-H, respectively (Figure 6). In addition, the slope allows determination of $\delta_{heterostack}$ or $\Delta_{heterostack}$ ($\delta_{free} - \delta_{heterostack}$). These Δ values are 0.18 ppm for C8-H and 0.28 ppm for C2-H. A cursory view of the X-ray structure of ethidium bromide-iodo-CpG[18] indicates greater overlap of the ethidium ring with the C2-H (i.e., pyrimidine) than with the C8-H (i.e., imidazole). This may explain why $\Delta_{heterostack}$ (C2-H) > $\Delta_{heterostack}$ (C8-H). Considering the fact that the K's were determined by the two widely different physical approaches, we consider the agreement between the numbers gratifying. To our best knowledge no other quantitative determination of ethidium bromide to 2′-deoxyadenosine binding has been reported in the literature. In 1 M NaCl dA-dT was reported to have a binding constant of 2×10^3 M^{-1}.[21] As our results were obtained at a rather low ionic strength (0.025 due to ethidium bromide only), supposedly the difference between our results and those of Bresloff and Crothers[22] would be even larger at the same ionic strength. In any case this $K_{heterostack}$ is rather large compared to, for example, tryptophan-coenzyme interactions previously studied in this laboratory[23] which was also an ion-dipole interaction as is the ethidium bromide-2′-deoxyadenosine one.

Conclusions

Our results demonstrate that the rotational barrier in syn-

anti isomerization in 2′-deoxyadenosine decreases on transferral of the nucleoside from a self-stacked environment to one involving heterostacking to either a tryptophan derivative (indole-3-acetic acid) or ethidium bromide. Obviously, one is tempted to speculate on the significance of such results. In the binding of intercalators to DNA the flexibility of the glycosyl dihedral angle must be severely restricted in any case. It is well established that intercalculation causes unwinding of DNA.[24] If the intercalator were to be located between the penultimate and terminal residues, ease of glycosyl bond rotation for the terminal residue is suggested. Equally interesting is the suggestion from our results that, when the purine nucleoside is bound to some receptor (typically a protein), the barrier to glycosyl conformational rotation is smaller than for the free (mostly self-stacked) nucleoside. Conformational changes around glycosyl C-N bonds upon binding to enzymes have already been suggested.[25]

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Protonic Reorganization and Substrate Structure in Catalysis by Serine Proteases[1]

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Received December 26, 1979

Abstract: Proton inventories (rate measurements in binary mixtures of protium and deuterium oxides) have been used to estimate the number of protons involved in hydrolytic catalysis by serine proteases with various substrates. Trypsin with the oligopeptide analogue BzPhe-Val-Arg *p*-nitroanilide as substrate produces an overall solvent isotope effect V_{H_2O}/V_{D_2O} of 4.30, and a proton inventory consistent with two-proton catalysis, with each proton generating an isotope effect of about 2.1. α-Chymotrypsin with the truncated substrates $AcTrpNH_2$ (acylation rate limiting) and 4-nitrophenyl 3-phenylpropanoate (deacylation rate determining) also produces apparent two-proton catalysis but with smaller overall isotope effects and skewed contributions from the two sites ($V_{H_2O}/V_{D_2O} = 1.90 \sim 1.69 \times 1.14$ for $AcTrpNH_2$ and $V_{H_2O}/V_{D_2O} = 2.85 \sim 1.85 \times 1.54$ for 4-nitrophenyl 3-phenylpropanoate). On the other hand, trypsin with the similar substrate BzArgOEt ($V_{H_2O}/V_{D_2O} = 3.03$) and thrombin with BzArgOEt ($V_{H_2O}/V_{D_2O} = 2.92$) give one-proton results, with deacylation presumably rate limiting in both cases. The minimal substrate *p*-nitrophenyl acetate with α-chymotrypsin ($V_{H_2O}/V_{D_2O} = 2.40$) and elastase ($V_{H_2O}/V_{D_2O} = 2.92$) shows one-proton catalysis for rate-determining deacetylation, while with trypsin the overall effect ($V_{H_2O}/V_{D_2O} = 1.38$) is too small to resolve the question of the number of active protons. Apparently, oligopeptide structure, leading to enzyme–substrate interactions at remote subsites as well as at the catalytic site in the catalytic transition state, is required to bring into action the full evolutionarily developed acid–base machinery of the serine proteases. If the structure is reduced to the point where only catalytic site interactions occur, the reliability of the acid–base machinery is much impaired, while, with minimal substrates, the enzyme acts either as a simple general catalyst or perhaps even as a nucleophilic catalyst. Compression of the distance across the catalytic hydrogen-bond chain of the active site as a consequence of remote-site interactions in the transition state, with relaxation of the enzyme structure occurring in their absence, is a reasonable mechanism for the coupling and decoupling of the protonic interactions by substrate structure.

The serine proteases[2-4] are enzymes, found at all levels of biological development, which have arrived at a common chemical solution to the catalytic problem of accelerating the hydrolysis of polypeptides to amino acids. They employ a double-displacement mechanism (eq 1), in which the eponymous serine is first acylated with expulsion of the amine leaving group (eq 1a) to form the acyl-enzyme intermediate; the acyl-enzyme then hydrolyzes in the second step (eq 1b) to give the carboxylate product.

$$ECH_2OH + R_1CONHR_2 \rightarrow ECH_2OCOR_1 + R_2NH_3^+ \quad (1a)$$

$$ECH_2OCOR_1 + H_2O \rightarrow ECH_2OH + R_1CO_2^- \quad (1b)$$

0002-7863/80/1502-3917$01.00/0