Solvent Effects on the Kinetics of the Interaction of 1-Pyrenecarboxaldehyde with Calf Thymus DNA

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The kinetics of the interaction of a fluorescent probe, 1-pyrenecarboxaldehyde, with calf thymus DNA has been studied in different water/alcohol mixtures (ethanol, 2-propanol, and ter-butanol) at 25 °C, by using the stopped flow technique. The kinetic curves are biexponential and reveal the presence of two processes whose rates differ by about 1 order of magnitude on the time scale. The dependence of the reciprocal fast relaxation time on the DNA concentration is linear, whereas the concentration dependence of the reciprocal slow relaxation time tends to a plateau at high DNA concentrations. The simplest mechanism consistent with the kinetic results involves a simple two-step series mechanism reaction scheme. The first step corresponds to the formation of a precursor complex, $(DNA/Py)_I$, while the second one corresponds to full intercalation of the pyrene dye between the DNA base pairs. The values of the rate constants of both steps decrease as water activity decreases. The results have been discussed in terms of solvation of the species and changes in the viscosity of the solution.

Introduction

The understanding of the mechanistic aspects by which small ligands interact with nucleic acids, producing different biological effects, has been a main topic of the scientific community in the past few decades. For instance, in the field of antitumor molecule design, it has been found that, for a drug to be efficient as a cancer therapeutic agent, a slow rate of dissociation from DNA is one of the most important properties.[1] Therefore, it seems clear that quantification of the kinetics of association/dissociation of small molecules to/from DNA is of diagnostic importance.

Water is an integral part of the DNA structure, since at least two hydration layers surround double-stranded DNA.[2] Hydration plays, for instance, an important role in both specificity and binding of protein to DNA.[3] Concerning drug−DNA interactions, the understanding of how DNA−solvent interactions influence the thermodynamics and kinetics of the binding of small ligands is of particular importance in order to improve drug design. Actually, some studies related to the effect of alcohol−water mixtures on the thermodynamics of dye binding to DNA have been previously performed,[4,5] in order to gain information on the factors that control these types of reactions. Moreover, despite the many efforts made in this direction, the mechanism of interactions of solvents themselves (alcohols and water) with biomolecules such as DNA is still not clear. Different points of view have been adopted to analyze this question. One of them considers the direct binding of the alcohol (and water) to the residues present in macromolecules.[6] Another point of view treats this problem as an indirect effect, involving changes in the properties of the water solvent caused by alcohols.[7] In this regard, the effects of alcohols on DNA have often been discussed as governed by a lowering of the dielectric constant of the solvent,[4] within a model that considers the solvent to be a continuous medium characterized by bulk properties such as the dielectric constant.[4] Others studies, using the osmotic stress method, have been carried out in order to examine the hydration changes that accompany biomolecular interactions. For instance, this technique has been used to study water uptake/release upon complex formation in DNA intercalation reactions[8] and groove binding reactions.[9] It thus seems clear that the analysis of solvent effects in DNA−ligand interactions is an interesting area that has been explored mainly by thermodynamic, structural and transport methodologies. By contrast, studies on solvent influence on the kinetics of DNA−ligand interactions, particularly concerning alcohols, are relatively scarce.[10] As a contribution to this field, we report in the present paper a thermodynamic and kinetic study of the influence of water−alcohol mixtures of different hydrophobic characteristics (ethanol, 2-propanol, and ter-butanol) on binding of a fluorescent intercalator, 1-pyrenecarboxaldehyde (PyCHO), to calf thymus DNA (CT-DNA). Pyrene and its derivatives are powerful probes in studying the molecular dynamics, structural organization, and polarity of assemblies in which hydrophobic environments are defined.[11] In this sense, numerous thermodynamic and structural studies on the binding to DNA of pyrene and its derivatives have been performed by using different techniques such as absorption,[12−15] fluorescence spectroscopy,[16] circular dichroism (CD),[13] and optical decoupling Raman spectroscopy.[12] The aim of this investigation is to analyze the solvent effects on the kinetics of the reaction of PyCHO with DNA, taking into account the solute−solvent interactions and the structural perturbations of DNA induced by the binding process.

Experimental Section

Materials. CT-DNA was purchased from Pharmacia and used without further purification. An agarose gel electrophoresis test

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using ethidium bromide indicated that the average number of base pairs per DNA molecule is above 10.000 bp.

Polynucleotide concentrations were determined spectrophotometrically from the molar absorptivity (6600 M^{-1} at 258 nm in order to have the DNA concentration in phosphate units).[17] The other reagents were all analytical grade and were used as purchased. Stock solutions of PyCHO were prepared by dissolving weighted amounts of the solid in the appropriate alcohol and keeping them in the dark. Working solutions were obtained by dilution with water of the stock solutions to reach the desired alcohol concentration in each case. The water used in the preparation of solutions had a conductivity of less than 10^{-6} S m^{-1}.

Methods

Kinetics. The kinetic experiments were performed at 25 °C by using a Biologic SF 300 stopped-flow instrument and monitoring the course of the reaction in the fluorescence detection mode. This detection mode was employed because the signal-to-noise ratio was found to be more favorable compared to the absorbance mode. The acquired signal was recorded on a PC and then analyzed by using the Jandell AISN software program. The dye concentration was 5×10^{-7} M. Preliminary spectroscopic measurements revealed that PyCHO does not self-aggregate at this concentration, thus ensuring that coupling between self-aggregation and binding processes does not occur. The DNA concentration was varied within 5×10^{-5} M and 1×10^{-3}M. All the kinetic experiments were performed under pseudo first-order conditions ([DNA] > 10[PyCHO]). Each experiment was repeated at least 10 times, and the relevant kinetic traces were accumulated in order to reduce the signal-to-noise ratio. The spread of time constants was found to be within 10%.

CD Spectra. CD spectra were recorder in a BioLogic Mos-450 spectropolarimeter. The measurements were performed in standard quartz cell of 1 cm path length in the 210−450 nm range. For each spectrum 5−10 runs were averaged at a constant temperature of 25 °C with a 5 min equilibration interval before each scan. All the spectra were recorded using fixed concentration of [DNA] (10^{-4} M) in the absence or in the presence of different concentrations of PyCHO ranging from 2.5×10^{-7} M to 1×10^{-6} M. The experiments were carried out in 25% ethanol (by weight).

Results and Discussion

PyCHO is a fluorescent probe that becomes less emissive upon binding to a DNA duplex. According to Cho et al., this singular circumstance arises from intercalation of the dye between the DNA base pairs.[12] We have previously performed a thermodynamic study of the binding of PyCHO to DNA in different alcohol media, [5b] and some of the results of that work will be considered here.

As regards the kinetic study of PyCHO binding to DNA, the kinetic curves were appropriately fitted by two exponentials, whose relaxation times, τ_1 and τ_2, differ by about 1 order of magnitude. An example of a typical kinetic experiment appears in Figure S1 (Supporting Information). The kinetic behavior of the system can be rationalized on the basis of the two-step series model depicted in Scheme I:

$$\mathrm{DNA} + \mathrm{Py} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (\mathrm{DNA/Py})_{\mathrm{I}} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (\mathrm{DNA/Py})_{\mathrm{II}} \qquad \text{(I)}$$

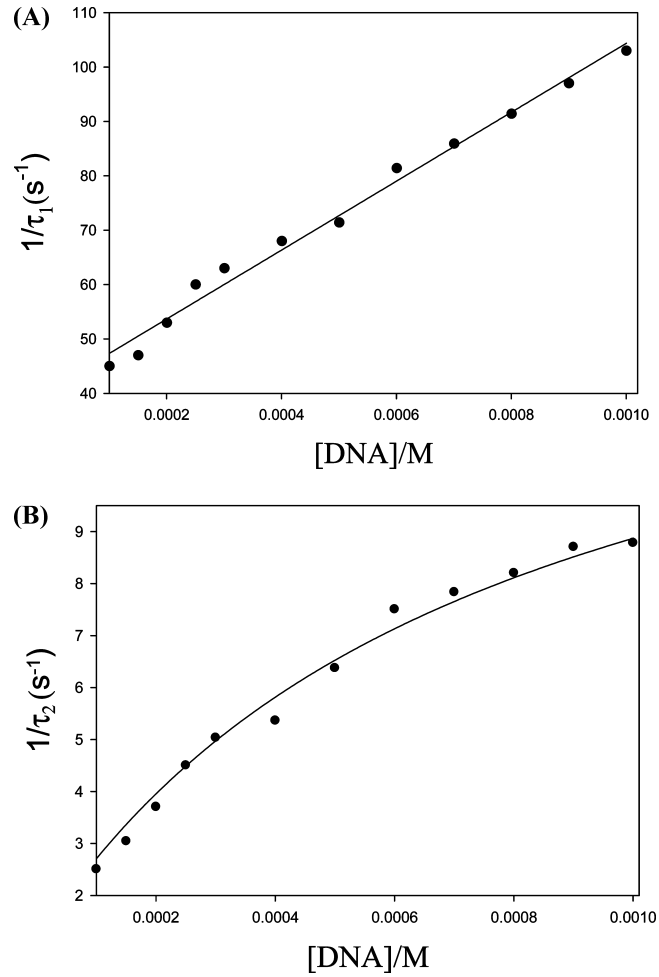

Figure 1. Reciprocal fast ($1/\tau_1$, A) and slow ($1/\tau_2$, B) relaxation times dependence on CT-DNA concentration at 25 °C in 16.7% 2-propanol (by weight, corresponding to $X_{(2\text{-propanol})} = 0.0566$).

where $(\mathrm{DNA/Py})_{\mathrm{I}}$ and $(\mathrm{DNA/Py})_{\mathrm{II}}$ represent two different DNA−dye complexes.

The dependence of the reciprocal of the fast relaxation time, $1/\tau_1$, on the DNA concentration is linear, according to eq 1.[18]

$$\frac{1}{\tau_1} = k_1[\mathrm{DNA}] + k_{-1} \qquad (1)$$

Thus, k_1 and k_{-1} can be obtained from the plot of $1/\tau_1$ versus the DNA concentration. An example of this kind of plot is shown in Figure 1A. Once k_1 and k_{-1} have been obtained from eq 1, the value of K_1 is calculated as k_1/k_{-1}.

On the other hand, the [DNA] dependence of the reciprocal slow relaxation time, $1/\tau_2$, tends to a plateau in agreement with eq 2.[18]

$$\frac{1}{\tau_2} = \frac{k_2 K_1[\mathrm{DNA}]}{1 + K_1[\mathrm{DNA}]} + k_{-2} \qquad (2)$$

The value of $K_1 = k_1/k_{-1}$ is introduced into eq 2 as a known parameter, while k_2 and k_{-2} are evaluated by data fit to eq 2 (Figure 1B). Once k_2 and k_{-2} have been obtained from eq 2, the value of K_2 is calculated as k_2/k_{-2}. The values of the rate and equilibrium constants of the first ($K_1 = k_1/k_{-1}$) and second ($K_2 = k_2/k_{-2}$) of the two steps of Scheme I are collected in Table

TABLE 1: Reaction Parameters for the Interaction of CT-DNA with PyCHO in Different Water/Alcohol Mixtures[a]

$X_{(ethanol)}$	$10^{-4}k_1$ ($M^{-1}s^{-1}$)	k_{-1} (s^{-1})	k_2 (s^{-1})	k_{-2} (s^{-1})	$10^{-3}K_1$ (M^{-1})	K_2
0.0202	16.6 ± 0.6	106 ± 3	72 ± 2	1.47 ± 0.15	1.56 ± 0.07	49 ± 5
0.0329	10.4 ± 0.3	82 ± 2	49 ± 2	1.30 ± 0.20	1.26 ± 0.05	37 ± 6
0.0507	6.7 ± 0.6	57 ± 3	28 ± 1	1.10 ± 0.21	1.19 ± 0.12	25 ± 5
0.0693	4.9 ± 0.2	38 ± 1	15 ± 1	0.91 ± 0.18	1.29 ± 0.06	16 ± 3
0.0891	3.7 ± 0.4	24 ± 2	8 ± 0.8	0.75 ± 0.13	1.54 ± 0.21	11 ± 2
0.1150	2.8 ± 0.2	13 ± 1	4 ± 0.4	0.55 ± 0.06	2.17 ± 0.23	5 ± 0.7
$X_{(2\text{-propanol})}$	$10^{-4}k_1$ ($M^{-1}s^{-1}$)	k_{-1} (s^{-1})	k_2 (s^{-1})	k_{-2} (s^{-1})	$10^{-3}K_1$ (M^{-1})	K_2
0.0220	13.4 ± 0.6	123 ± 3	102 ± 4	2.17 ± 0.30	1.09 ± 0.06	47 ± 7
0.0291	10.4 ± 0.5	99 ± 3	66 ± 4	1.82 ± 0.22	1.05 ± 0.06	36 ± 4
0.0382	8.2 ± 0.6	75 ± 3	48 ± 2	1.60 ± 0.21	1.10 ± 0.09	30 ± 4
0.0566	5.8 ± 0.4	41 ± 1	15 ± 0.4	1.13 ± 0.18	1.44 ± 0.11	13 ± 2
0.0717	4.9 ± 0.5	24 ± 3	5 ± 0.2	0.84 ± 0.09	1.98 ± 0.32	5 ± 0.7
$X_{(ter\text{-}butanol)}$	$10^{-4}k_1$ ($M^{-1}s^{-1}$)	k_{-1} (s^{-1})	k_2 (s^{-1})	k_{-2} (s^{-1})	$10^{-3}K_1$ (M^{-1})	K_2
0.0126	17.2 ± 0.9	121 ± 5	96 ± 3	3.38 ± 0.44	1.40 ± 0.09	28 ± 4
0.0207	11.9 ± 0.4	106 ± 2	77 ± 5	3.30 ± 0.70	1.15 ± 0.04	23 ± 5
0.0271	9.5 ± 0.5	94 ± 3	63 ± 5	3.26 ± 0.61	1.02 ± 0.06	19 ± 4
0.0362	7.5 ± 0.5	77 ± 3	45 ± 2	3.14 ± 0.28	0.95 ± 0.07	14 ± 1
0.0526	5.5 ± 0.2	48 ± 1	13 ± 1	2.99 ± 0.27	1.14 ± 0.05	4 ± 0.5

[a] 25 °C; X is the molar fraction of alcohol.

1 for different conditions of alcohol type and concentration. Considering the equilibrium constant (K) of the global process shown in Scheme II,

$$\mathrm{DNA} + \mathrm{Py} \overset{K}{\leftrightarrows} (\mathrm{DNA/Py})_{\mathrm{Tot}} \qquad \text{(II)}$$

where $(DNA/Py)_{Tot} = (DNA/Py)_I + (DNA/Py)_{II}$, it turns out that eq 3 holds.

$$K = K_1(1 + K_2) \qquad (3)$$

The values of K derived from the combination of the kinetic data by means of eq 3 agree with those obtained from the static fluorescence measurements previously performed[5b] (Table 2), thus supporting the validity of the reaction Scheme I.

Concerning the intermediate complex $(DNA/Py)_I$, it should be noted that early investigations on nucleic acid−dye interactions with positively charged ligands[19] demonstrated that electrostatics provide an important contribution to the stabilization of the intermediate complex, while stacking interactions were principally responsible for the stability of the final intercalated complex. More recently it has been shown that nonelectrostatic interactions also contribute remarkably to the stabilization of the intermediate bound form.[20] In the present system, being that the dye is uncharged, the electrostatic interaction could be neglected and the stability of $(DNA/Py)_I$ is entirely committed to nonelectrostatic forces acting between the aromatic surface of PyCHO and the DNA grooves. However, it could also be that, in the first step of the process shown by Scheme I, the ligand intercalates partially, thus inducing only a moderate conformational torsion of the double helix. Full ligand penetration between base pairs to form $(DNA/Py)_{II}$ is accomplished in the second step.

On the basis of these considerations, it seems reasonable that hydration effects and solute−solvent interaction would be of importance in the first step of the reaction. Concerning the variation of k_1 and k_{-1} with the alcohol concentration, Table 1

TABLE 2: Overall Equilibrium Binding Constants of Complex Formation between PyCHO and CT-DNA in Different Water/Alcohol Mixtures[a]

$X_{(ethanol)}$	$10^{-3}K^b$ (M^{-1})	$10^{-3}K^c$(M^{-1})
0.0202	78 ± 9	74 ± 5
0.0329	48 ± 8	55 ± 4
0.0507	31 ± 7	
0.0693	22 ± 4	5 ± 1
0.0891	19 ± 4	
0.1150	13 ± 2	9 ± 1
$X_{(2\text{-propanol})}$	$10^{-3}K^b$ (M^{-1})	$10^{-3}K^c$ (M^{-1})
0.0220	52 ± 8	61 ± 5
0.0291	39 ± 4	36 ± 5
0.0382	34 ± 4	25 ± 4
0.0566	20 ± 4	20 ± 3
0.0717	12 ± 3	
$X_{(ter\text{-}butanol)}$	$10^{-3}K^b$ (M^{-1})	$10^{-3}K^c$ (M^{-1})
0.0126	41 ± 6	41 ± 3
0.0207	28 ± 6	37 ± 4
0.0271	20 ± 4	
0.0362	14 ± 2	9 ± 1
0.0526	6 ± 0.8	8 ± 1

[a] 25 °C; X is the molar fraction of alcohol. [b] From the combination of the kinetic parameters of Table 1 according to eq 3. [c] From fluorescence titration data given in a previous work,[5b] adjusted to the Hildebrand and Benesi model.

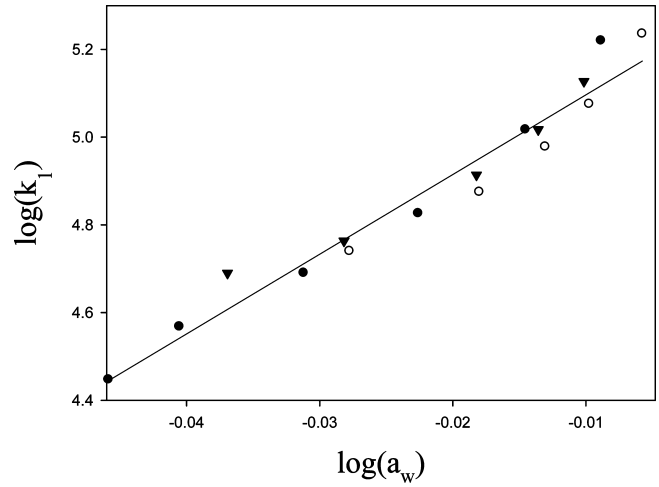

Figure 2. Plot of $\log(k_1)$ versus $\log(a_w)$ for different water/alcohol mixtures (25 °C), where k_1 is the forward kinetic constant defined in Scheme I and a_w represents the water activity of the solution. Ethanol (●), 2-propanol (▼), and ter-butanol (○).

shows that there is a correlation between these constants and the corresponding activity[21] of water (a_w) in the alcohol mixtures. The values of the logarithm of the forward kinetic constant (k_1), when plotted against $\log(a_w)$, lie on the same straight line, independently of the type of alcohol used (Figure 2). Similar behavior is displayed by the backward rate constant k_{-1} (Figure 3). These findings suggest that water activity (or some parameter directly related to it) plays an important role in the first step of the reaction scheme and gives support to the importance of the hydration of the species involved in this binding step. Concerning this issue, it should be noted that intense efforts are being made in measuring the total hydration changes that accompany ligand−DNA interaction using the osmotic stress method.[8,9,22]

Changes in k_1 and k_{-1} can be explained on the basis of solvent−solute interactions. According to the Bronsted argument,[23] it turns out that

$$k_1 = k_1^0 \frac{\gamma_{DNA}\gamma_{Py}}{\gamma_{\neq}} \quad (4)$$

$$k_{-1} = k_{-1}^0 \frac{\gamma_{(DNA/Py)_I}}{\gamma_{\neq}} \quad (5)$$

where k_1^0 is the rate constant in the reference state (we assigned such a reference state to the reaction in water), and γ_{DNA}, γ_{Py}, $\gamma_{(DNA/Py)}$, and $\gamma_{\neq}$ are, respectively, the activity coefficients of DNA, PyCHO, $(DNA/Py)_I$, and transition state. According to eqs 4 and 5, changes of the activity coefficients of the reacting species and also of the transition state, as a consequence of solute−solvent interactions, would be reflected in changes of k_1 and k_{-1}. In terms of the transition-state theory,[24] the rate constant k_1 measures the difference between the standard chemical potentials of the reactants and the transition state. It is difficult to predict these changes, as the alcohol concentration varies, because it is necessary to quantify the activity coefficients of the reactants and transition state for each alcohol concentration. However, if the activity coefficient of PyCHO could be evaluated in the different mixtures, one could compare the changes in the activity coefficient ratio $\gamma_{DNA}/\gamma_{\neq}$ for the direct step using eq 6:[5b]

$$(k_1)_{corr} = \frac{k_1}{\gamma_{Py}} = k_1^0 \frac{\gamma_{DNA}}{\gamma_{\neq}} \quad (6)$$

The values of γ_{py}, were estimated by means of solubility measurements of the probe in different alcohol media.[5b] The activity coefficient of PyCHO (Table S1, Supporting Information) decreases by about 2 orders of magnitude as the alcohol concentration increases by a factor of 5. On the contrary, Table 1 shows that k_1 decreases as the concentration of the different alcohols increases. This result can likely be ascribed to stabilization of PyCHO due to the presence of the alcohol in the medium. In fact, when the values of k_1 are corrected for this effect using eq 6, it turns out that the values of $(k_1)_{corr}$ do increase as the alcohol molar fraction increases (Figure 4).

Concerning the reverse process, similarly to what occurs with k_1, Table 1 shows that k_{-1} decreases with increasing alcohol concentration. That is, dissociation of PyCHO from DNA is hindered by the alcohol. It is clear that our results imply a decrease of the $\gamma_{(DNA/Py)}/\gamma_{\neq}$ ratio as the alcohol level is raised.

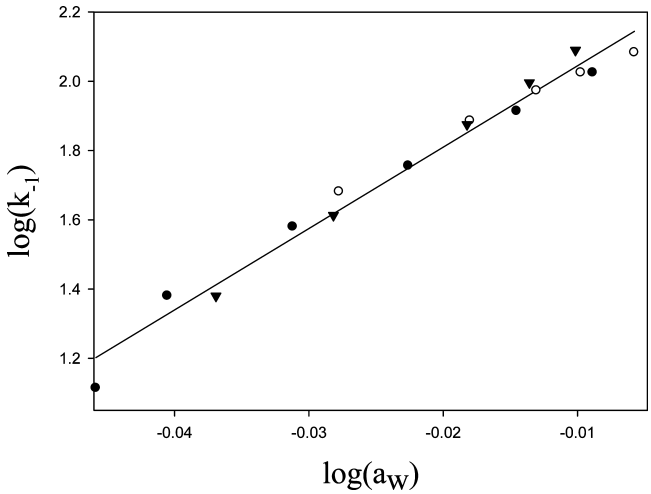

Figure 3. Plot of $\log(k_{-1})$ versus $\log(a_w)$ for water alcohol mixtures (25 °C): ethanol (●), 2-propanol (▼), and ter-butanol (○).

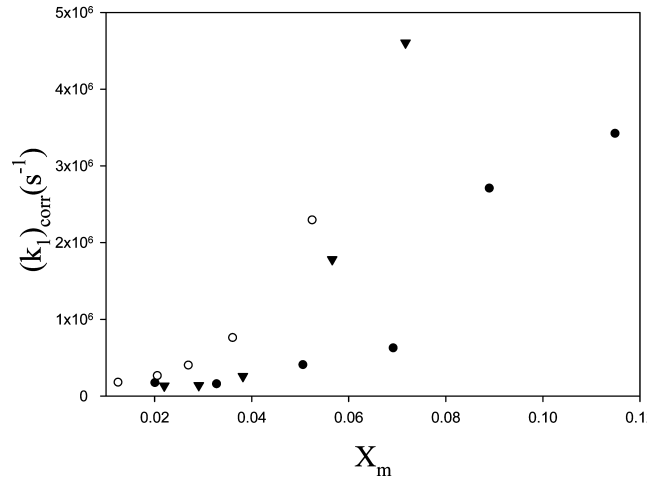

Figure 4. Plot of corrected forward kinetic constant, $(k_1)_{corr}$, as a function of molar fraction of alcohol, X_m, for different water/alcohol mixtures (25 °C). Ethanol (●), 2-propanol (▼), and ter-butanol (○).

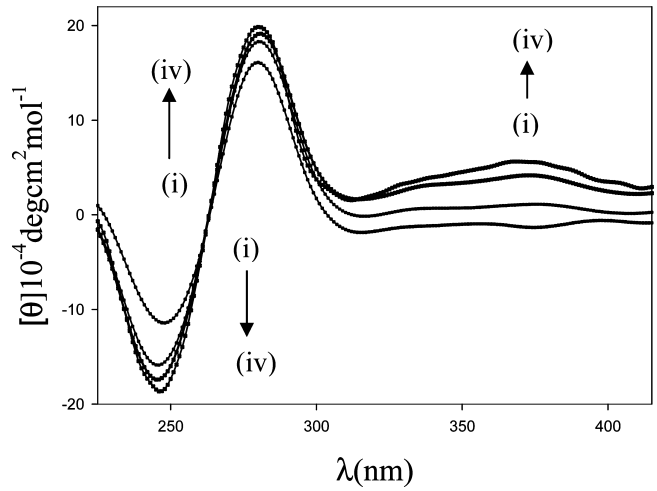

Figure 5. CD spectra of the CT-DNA/PyCHO system at 25 °C and 25% EtOH (by weight) under conditions of different dye content. [CT-DNA] = 1×10^{-4} M; [PyCHO] = 0 (i), 2.5×10^{-7} (ii), 5.0×10^{-7} (iii), and 1.0×10^{-6} M (iv).

Concerning the variations of k_2 and k_{-2} with the alcohol content, one could reasonably assume that a structural perturbation accompanies the transformation of $(DNA/Py)_I$, formed in the first step, toward the conformation of the intercalated complex, $(DNA/Py)_{II}$, and its reverse process, respectively. This perturbation is necessary for the intercalation of the probe into the double helix.[12] In fact, in order to allow the intercalation, base pairs must separate (unstack) vertically and the sugar puckering at the 5′ side of intercalation should change from C2′-endo to C3′-endo.[2b] Furthermore, dye desolvation is necessary for intercalation to occur, [25] and the DNA−solvent interactions in the proximity of the intercalation site will be affected by the unwinding of the double helix and the increased separation of the phosphate groups. To properly identify these perturbations of the secondary structure of DNA due to the intercalation of the probe, CD experiments were performed. The CD spectrum of DNA (Figure 5) shows a positive peak at approximately 278 nm and a negative peak at 247 nm. Structure alterations of DNA caused by its interaction with PyCHO should be reflected in changes of the intrinsic CD spectrum.[26] Increasing concentrations of PyCHO lead to remarkable perturbations of

the positive and negative bands. The intensities of both bands increase; meanwhile, no considerable shift in their positions appears as PyCHO is added to the solution. These changes reflect the local unwinding of the helical backbone and the changes in the relative orientation of the bases to accommodate the intercalation probe within base pairs.[27] On the other hand, further evidence of intercalation of PyCHO is provided by the changes occurring when the induced CD of the dye could be observed (Figure 5).[28]

A study on the ethidium bromide intercalation into polyd(G-C) by McGregor and Clegg,[10a] was focused on the influence of the solvent viscosity on the rate of binding. The authors found that the kinetic constants for the association process decreases proportionally to the solvent viscosity, and interpreted their results in terms of diffusion controlled binding of ethidium to a minority form of DNA. The unwinding/winding of the double helix, necessary to host/exclude a drug between base pairs, requires both large-scale diffusion of the polymer chain and local reconfigurations of the sugar puckering and slide chains of the DNA. Kramer's model[29] for reaction rates provides a theoretical explanation to understand diffusion-controlled barrier-crossing processes. Kramer's theory asserts that the rate constant k_j is proportional to $\gamma^{-1}\exp(-\Delta G_j^{\ddagger}/RT)$ where $\Delta G_j^{\ddagger}$ is the free activation energy barrier and γ is a friction parameter. Extensions of Kramers theory have been used to describe the folding/unfolding reactions of proteins[30] and in general to gain insight into the dynamics of conformational changes in macromolecules.[31] One such extension[30c,32] is the inclusion of an adjustable parameter, ξ, which, together with the bulk solvent viscosity (η_S), provides the dependence on viscosity of the diffusional rate constant of a monomolecular conformational change of a protein in terms of eq 7:

$$k_j = \frac{v}{\eta_s + \xi}\exp(-\Delta G_j^{\ddagger}/RT) \tag{7}$$

where $\Delta G^{\ddagger}$ is the activation free energy barrier separating the two protein conformations, and v is a constant of proportionality. Applying the above argument to the conformation interchange $(DNA/PyCHO)_I \leftrightarrows (DNA/PyCHO)_{II}$, one could use eq 7 to interpret the dependence of k_2 and k_{-2} on the alcohol content as shown below.

It is known that cosolvents such as sucrose and others sugars, polyols, and methylamines act as ligands that stabilize the native structure of macromolecules in solution.[33] These ligands interact with the macromolecules according to the weak interaction model.[34] Applying this model to the interaction of alcohols with DNA, one can assume that the change in free energy barrier of the conformational change induced by intercalation of PyCHO into DNA is linearly dependent on the alcohol molar fraction, X_m.[34]

$$\Delta G_j^{\ddagger} = (\Delta G_j^{\ddagger})^0 + aX_m \tag{8}$$

In this equation, $(\Delta G_j^{\ddagger})^0$ represents the energy barrier in the absence of cosolvent, and the a parameter is evaluated from the slope of the plot of the logarithm of the rate constant in the presence of alcohol versus cosolvent concentration, X_m. This slope corresponds to $-a/2.303RT$. Considering the dependence of the system viscosity on the alcohol concentration as well, in the form[35] $\eta_S = \eta_S^0 + bX_m$, introduction of eq 8 into eq 7 yields eq 9:

$$k_j = \frac{v}{(\eta_S^0 + bX_m) + \xi}\exp - \frac{((\Delta G_j^{\ddagger})^0 + aX_m)}{RT} \tag{9}$$

In the present study, the viscosity dependence on the solvent composition was obtained from the literature, [35] and it was found that it is linear in the range of study. In the absence of any added cosolvent, eq 7 can be written as follows:

$$k_{j,0} = \frac{v}{\eta_S^0 + \xi}\exp - \frac{(\Delta G_j^{\ddagger})^0}{RT} \tag{10}$$

In the presence of alcohol, the combination of eqs 9 and 10 yields eq 11:

$$k_j = \frac{k_{j,0}(\eta_S^0 + \xi)}{(\eta_S^0 + bX_m) + \xi}\exp - \frac{aX_m}{RT} \tag{11}$$

This equation relates the changes in kinetic parameters with the changes of alcohol concentrations and it also permits one to get an estimation, on the basis of the Kramer theory, of the influence of local viscosity on both k_2 and k_{-2}. As an example, a plot of $k_2/k_{2,0}$ and $k_{-2}/k_{-2,0}$ as a function of alcohol concentration is shown in Figure 6, for water−ethanol mixtures, and the data fit well to eq 11. Similar correlations have been found for the other alcohols. The values of the parameter ξ obtained from the fit of the experimental data to eq 11 were 1, 4, and 9 cP for ethanol, 2-propanol, and ter-butanol, respectively. Effective viscosity can be obtained for each alcohol concentration:[35]

$$\eta^{\text{effective}} = \eta_S + \xi \tag{12}$$

It turns out that the effective viscosity changes in the order ter-butanol > 2-propanol > ethanol, the trend being driven by the ξ values. In this sense, as the hydrophobic character of the alcohol increases (ter-butanol > 2-propanol > ethanol), the effective viscosity is higher. This result probably reflects the

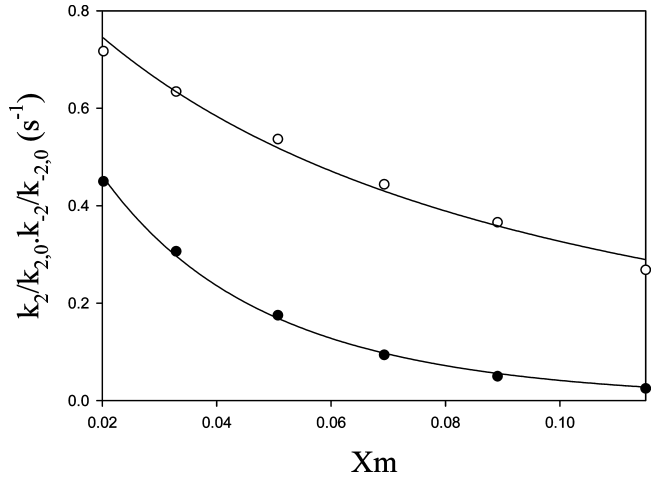

Figure 6. Relative forward $k_2/k_{2,0}$ (●) and backward $k_{-2}/k_{-2,0}$ (○) rate constants corresponding to the second step of Scheme II (eq 10) versus X_m, the molar fraction of ethanol (25 °C). The forward kinetic constant was represented relative to the value in the absence of viscogen ($k_{2,0} = 160\ s^{-1}$) and then adjusted to eq 11; for the backward kinetic constant k_{-2}, the value of $k_{-2,0} = 2.05\ s^{-1}$ was used.

relative importance of the size of alcohol and the hydrophobic effect on the kinetics of this reaction.

Conclusions

In conclusion, a two-step series mechanism has been proposed for the reversible binding of PyCHO to DNA. According to the results, it seems that the effect of the solvent on the mechanism of this reaction is controlled not only by the dielectric constant of the solvent but, to some extent, by the structural character of the solvent as well. The first step of the reaction, which implies the formation of a precursor complex $(DNA/Py)_I$, is governed by the solvation interactions. A linear dependence has been observed for both the direct and reverse kinetic constants of this step on water activity. The second step has been interpreted as full ligand penetration between base pairs to form the final intercalated complex $(DNA/Py)_{II}$. The variations of direct and backward kinetic constants, k_2 and k_{-2}, of the second step are found to be dependent on the viscosity of the solvent in terms of the Kramer theory.

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Supporting Information Available: Activity coefficients of PyCHO corresponding to different concentrations of alcohols; electrophoresis of CT-DNA against a ladder in agarose gel using ethidium bromide; stopped-flow relaxation curves for the CT-DNA/PyCHO system. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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