# Deoxyribonucleic Acid Bifunctional Intercalators: Kinetic Investigation of the Binding of Several Acridine Dimers to Deoxyribonucleic Acid†

Nicole Capelle,‡ Jacques Barbet,§ Philippe Dessen,‖ Sylvain Blanquet,‖ Bernard P. Roques,§ and Jean-Bernard Le Pecq*,‡

ABSTRACT: Kinetical investigation of the binding to DNA of several acridine dimers with chains of various length was carried out with the purpose of estimating the binding affinities of these drugs. Stopped-flow experiments have led to the determination of on-rate constants for the complexation reaction ($\simeq 3 \times 10^7$ $M^{-1}$ $s^{-1}$) which are independent of the studied dimer. Owing to the dependence of the fluorescence properties of DNA-bound acridine dimers with DNA sequence, off-rate constants were estimated from exchange experiments between poly[d(A-T)·d(A-T)] and GC-rich *Micrococcus luteus* DNA. These off-rate constants were found to be very small for all diacridines. Together with the on-rate constants, they provided affinity constants for poly[d(A-T)·d(A-T)], which can be as large as $2 \times 10^{11}$ $M^{-1}$ (at pH 5.0 in 0.1 M $Na^+$) for one of the derivatives. For one acridine dimer, the rate of exchange increased with the concentration of the displacing DNA. This phenomenon has been interpreted as resulting from the formation of a transient ternary complex between the dye and two DNA molecules. This led to a direct ligand transfer phenomenon. But in the case studied here, the ternary complex was more stable than the binary complex; hence, the direct ligand transfer led to a slower rate of exchange. This behavior could be eliminated by previous sonication of the displacing DNA. The effects of pH and ionic strength have been investigated. A dramatic decrease of the affinity at high pH and/or ionic strength is observed. At pH values greater than 7.0, the dimers are folded with the two acridine rings stacked. Under these conditions, unstacking of the dimer becomes the rate-limiting step for the association to DNA.

Since Lerman (1961) first proposed that acridine binds to DNA by intercalation between base pairs, many related compounds have been found able to bind to nucleic acids according to this process. Recently, this binding was characterized at atomic resolution (Tsai et al., 1975; Sakore et al., 1977; Jain et al., 1977). Several substances of interest for the treatment of malignant and parasitic diseases belong to this class of compounds. In some cases, it could be shown that the pharmacological properties of these drugs are related to their DNA binding affinity (Le Pecq et al., 1974; Di Marco et al., 1976). It is therefore of interest to prepare and study new molecules which could bind to nucleic acids with affinities comparable to those of repressor and RNA polymerase for DNA.

As a first attempt, we have prepared and studied several dimeric molecules which can potentially bisintercalate into DNA (Barbet et al., 1975, 1976; Le Pecq et al., 1975; Roques et al., 1976a,b; Butour et al., 1978; Gaugain et al., 1978). Similar molecules have also been prepared by Chen et al. (1978), Cain et al. (1978), Dervan & Becker (1978), and Kuhlmann et al. (1978). In such molecules the binding free energy of each subunit may add up, provided that entropy factors not be too unfavorable. Under these conditions, the binding constant of bisintercalators could theoretically become as large as $10^{12}$ or $10^{13}$ $M^{-1}$. Indeed, in preliminary studies (Le Pecq et al., 1975) a large affinity of acridine dimers for DNA was observed. As measurement of the equilibrium between free and bound molecules becomes inaccurate for reactants concentrations smaller than $10^{-8}$ M, it could only be concluded that the binding affinity of acridine dimers to DNA was larger than $10^8$ $M^{-1}$. In this case, the study of the kinetics of the interaction of these compounds with DNA leads directly to the estimation of their binding affinity and to further characterization of the DNA binding process.

From earlier works on actinomycins (Müller & Crothers, 1968; Crothers, 1971), it is known that their biological activity is better related to a low dissociation rate from DNA than to a high affinity. Inhibition of DNA-dependent RNA polymerase by these intercalating drugs was studied. The extent of inhibition was shown to correlate more satisfactorily with the kinetical dissociation rate from DNA rather than with the binding constant. It is therefore of interest to compare this rate in the case of the various dimeric intercalating drugs under study.

DNA-bound diacridine molecules have fluorescence parameters depending on DNA sequences (Le Pecq et al., 1975). This makes them particularly suitable for stopped-flow kinetics. In particular, the exchange of dyes can be monitored between DNAs of different base composition with concomitant variations of their fluorescence.

This work presents a kinetic investigation of the association of several acridine dimers with poly[d(A-T)·d(A-T)]. Exchange of the bound dyes between DNA molecules of different base composition has also been studied. For one diacridine molecule it is observed that the rate of exchange increases with the competitor DNA concentration, instead of occurring at a constant rate. This result can be best accounted for by direct

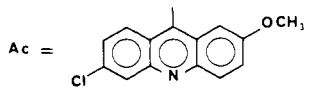
† From the Laboratoire de Physico-Chimie Macromoléculaire, Institut Gustave-Roussy, 94800 Villejuif, France, the Departement de Chimie Organique, Université René Descartes, 4, Avenue de l'Observatoire, 75006 Paris, France, and the Laboratoire de Biochimie, Ecole Polytechnique, 91120 Palaiseau, Cedex, France. *Received January 12, 1979.* Support of this research through grants from Université Pierre et Marie Curie (Paris VI), Université René Descartes (Paris V), CNRS, INSERM, Délégation à la Recherche Scientifique et Technique, and Fondation pour la Recherche Médicale is gratefully acknolwedged.

‡ Present address: Laboratoire de Physico-Chimie Macromoléculaire (LA No. 147 du CNRS et U 140 de l'INSERM), Institut Gustave-Roussy 94800, Villejuif, France.

§ Present address: Départment de Chimie Organique (ERA No. 613 du CNRS), UER des Sciences Pharmaceutiques et Biologiques, 4, Avenue de l'Observatoire, 75270, Paris, Cedex 06, France.

‖ Present address: Laboratoire de Biochimie (LA No. 240 du CNRS), Ecole Polytechnique, 91120 Palaiseau, Cedex, France.

0006-2960/79/0418-3354$01.00/0 © 1979 American Chemical Society



AcMo = Ac – NH – $(CH_2)_3$ N $(CH_3)_2$

AcDi 1 = Ac – NH – $(CH_2)_3$ NH $(CH_2)_4$ NH $(CH_2)_3$ – NH – Ac

AcDi 2 = Ac – NH – $(CH_2)_3$ NH $(CH_2)_4$ – NH – Ac

AcDi 3 = Ac – NH – $(CH_2)_3$ NH $(CH_2)_3$ – NH – Ac

FIGURE 1: Chemical structures of acridine monomer and dimers.

transfer of the bifunctional dye between two DNA molecules through an intermediary complex in which the dye binds to both DNA molecules. This phenomenon of direct transfer has been assumed to play an important role in increasing the binding rate of regulatory proteins to their DNA specific sites (for discussion and review, see Von Hippel et al., 1975; Jovin, 1976). Recent studies emphasize the bifunctional character of the ligands which exhibit direct transfer (Bresloff & Crothers, 1975; Blanquet et al., 1976; Giacomoni et al., 1977). Diacridine molecules might therefore represent a valuable model for the illustration of a direct transfer reaction.

## Materials and Methods

### *Chemicals*

Alternating poly[d(A-T)·d(A-T)] was obtained from Boehringer. DNAs from *Micrococcus luteus* and *Clostridium perfringens* were purchased from Sigma and further purified by phenol extraction. *Micrococcus luteus* DNA was sonicated under nitrogen at 4 °C as described elsewhere (Le Pecq et al., 1975). The synthesis and the conformations of the studied acridine derivatives have been described earlier (Barbet et al., 1975, 1976) and their structures are shown in Figure 1. Sedimentation coefficients of DNAs under study were measured by analytical sedimentation at 20 °C. Their values were respectively 21 S for poly[d(A-T)·d(A-T)], between 18 and 20 S according to the sample for *Micrococcus luteus* and *Clostridium perfringens* DNA, between 6 and 7 S for sonicated DNA.

### *Methods*

(*1*) *Exchange Kinetics.* It has been shown that two of the acridine derivatives, AcDi 1[1] and AcDi 2, can bisintercalate into DNA (Le Pecq et al., 1975). These acridine dimers become fluorescent only when they bind to DNA sequences made up of four consecutive AT base pairs. This property enables the easy determination of the dissociation rate of dye:DNA complexes. First the dye is preincubated with DNA I for at least 24 h; subsequently, the equilibrium is perturbed by the addition of an excess amount of the competing DNA II (richer than DNA I in GC or AT base pairs); the system relaxes to its new equilibrium, therefore providing a relaxation time associated with the variation of fluorescence. Fluorescence is measured at 20 °C with a photon counting spectrofluorometer built in this laboratory. Counts are accumulated as a function of time in a multichannel analyzer, Intertechnique SA 41 Didac 400. Excitation and emission wavelengths are 460 and 520 nm ($\Delta\lambda$ 10 nm), respectively.

For composite relaxations, a mean exchange rate is calculated according to Schwarz (1968) as the arithmetical mean of rate constants weighted by respective amplitudes.

(*2*) *Stopped-Flow Fluorescence.* Rapid mixing experiments are performed in a Durrum-Gibson stopped-flow spectrometer equipped with fluorescence detection. Excitation at 470 nm of the acridine fluorescence is ensured by a 450-W Osram xenon lamp followed by a Jobin–Yvon HRS2 monochromator. The slit width of the excitation monochromator is 1.5 mm ($\Delta\lambda$ 5 nm). The observation cell is a quartz tube of 2-mm diameter and 20-mm length. The emission is observed at 90° after passing through MTO A 538b and Corning 3.69 filters. The dead time of the apparatus is less than 2.5 ms. Relaxations are obtained by 1:1 mixing of 0.2 mL volume of reactants. The analogical output of the stopped-flow amplifier is stored after analogue-digital conversion (12 bits) and directly analyzed in a Nova 2 computer equipped with 16K words (16 bits). One thousand experimental points per relaxation are stored and then monitored under computer control by a programmed timer. Programmation of the timer enables data storage with variable sequential frequences. The experimental points are then reduced to a maximum of 50 points by using a polynomial least-squares regression. The reduced data are furthermore analyzed by a multinear iterative regression, thus providing the parameters for the relaxations. Finally, the stored experimental data are superimposed graphically to their fitted relaxations on an X–Y recorder.

(*3*) *Equilibrium Binding Constant Measurements.* These measurements are performed by using the photon counting spectrofluorometer as for exchange kinetic experiments. Fluorescence intensities of solutions containing dye plus DNA and dye alone of the same dye concentration are measured at various ionic strengths. The concentrations of bound and free dyes are computed according to Le Pecq & Paoletti (1967). Binding constants of the acridine monomer (AcMo) are calculated from Scatchard plots according to Mc Ghee & Von Hippel (1974).

In the case of acridine dimers, several types of binding coexist (Le Pecq et al., 1975). The binding constants could only be determined at high ionic strength according to Gaugain et al. (1978) by using the treatment of Mc Ghee & Von Hippel (1974).

(*4*) *Equilibrium dialysis experiments* are performed in three-chamber Teflon dialysis cells (Oriel, Paris, France). The two outer chambers are filled with 0.8 mL of 17 $\mu$M solutions of poly[(d(A-T)·d(A-T)] and of *Micrococcus luteus* DNA each. The inner chamber is filled with 0.8 mL of the dye solution. The filled dialysis cells are equilibrated for 24 h in a thermostated bath (23 °C) with slow agitation. Finally, the dye concentrations are determined spectrofluorometrically ($\lambda_{ex}$ 420 nm; $\lambda_{em}$ 500 nm) after 1:1 dilution with 0.5% acetic acid in dimethyl sulfoxide according to Müller et al. (1973).

## Results

(*1*) *Association Kinetics.* Aminoacridines and diacridines bind to poly[d(A-T)·d(A-T)] with a strong concomitant exaltation of their fluorescence. After rapid mixing of the two solutions of dye and of poly[d(A-T)·d(A-T)], a time-dependent increase of fluorescence is observed which reflects the formation of the intercalation complex (Figure 2). This reaction can be fitted in with the pseudo-first-order approximation as poly[d(A-T)·d(A-T)] is present in large excess with respect to the dye. The value of $r$, the ratio of bound dye to base pair, is kept small and constant ($r \simeq 0.02$). Under these conditions, the rate of dye binding to the DNA isolated sites is measured and the anticooperativity of the binding (Crothers, 1968) can therefore be neglected. The number of DNA sites equals the number of DNA base pairs.

The relaxations obtained with the acridine monomer (AcMo) and the acridine dimers AcDi 1, AcDi 2, and AcDi

[1] Abbreviations used: AcMo, acridine monomer; AcDi 1, AcDi 2, and AcDi 3, acridine dimers no. 1, 2, and 3, respectively (structures are shown in Figure 1).

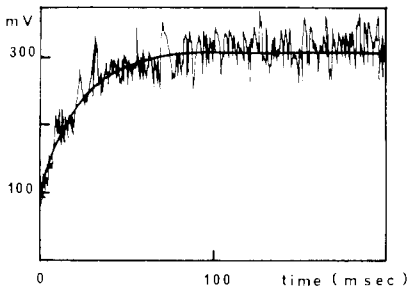



FIGURE 2: Relaxation for the formation of the poly[d(A-T)·d(A-T)]:AcDi 1 complex at 20 °C after 1:1 mixing of a solution containing $2 \times 1.86$ $\mu$M DNA base pairs in sodium acetate, 0.2 M (pH 5.0) with a solution containing $2 \times 0.048$ $\mu$M AcDi 1 in the same buffer. The rise time is 0.5 ms. The relaxation fits a single exponential with an amplitude of $0.225 \pm 0.016$ V and a rate constant equal to $52.3 \pm 5.4$ $s^{-1}$.

3 fit single exponentials as expected for a single equilibrium between dye (X) and DNA (P) with P $\gg$ X according to

$$P + X \underset{k_d}{\overset{k_a}{\rightleftharpoons}} PX$$

The observed rate constant ($k_{obsd}$) thus fits the relation:

$$k_{obsd} = k_a P_0 + k_d \quad (1)$$

($P_0$ being the molar concentration of poly[d(A-T)·d(A-T)] expressed in base pairs).

In all cases the rate constant, $k_{obsd}$, varies linearly with the poly[d(A-T)·d(A-T)] concentration as expected for a single bimolecular reaction under pseudo-first-order approximation. Rate constant variations as a function of DNA concentration are summarized in Figure 3. The values of the statistical association rate constants, $k_a$ and $k_d$, are given in the legend of Figure 3. It must be noted that the extrapolated values of the observed rate constants to zero DNA concentration for acridine dimers are close to zero with standard deviations precluding the determination of dissociation rates, $k_d$. By contrast, for the monomer AcMo due to the larger value of the dissociation constant, both $k_a$ and $k_d$ can be determined (Figure 3). As this point, it can be noted that the association rate constants for the monomer and for each studied acridine dimer are of the same order; this could indicate that the association of dyes with DNA is a diffusion-controlled process with identical statistical factors for the dimers as well as for the monomer.

*(2) Binding Constant of AcMo for Poly[d(A-T)·d(A-T)].* The intrinsic binding constant of AcMo was measured at equilibrium by fluorescence titration in order to compare it with the ratio of the kinetic rate constants, $k_a/k_d$. The variation of the intrinsic equilibrium affinity constant $K$ of AcMo for poly[d(A-T)·d(A-T)] as a function of ionic strength is shown (Figure 9). The dependence on ionic strength indicates the participation of two electrostatic interactions as will be discussed later. The close agreement between the values of the ratio $k_a/k_d$ and that of the intrinsic binding constants determined at equilibrium suggests that no major rearrangements of the AcMo–poly[(dA-T)·d(A-T)] complex are taking place.

*(3) Kinetics of Exchange of Acridines between Poly[d(A-T)·d(A-T)] and Micrococcus luteus DNA.* As shown above, the dissociation rate constants $k_d$ of diacridines from DNA could not be determined from kinetics measurements because of their low values (Figure 3). However, these rate constants can be obtained from exchange kinetics between two different DNAs.

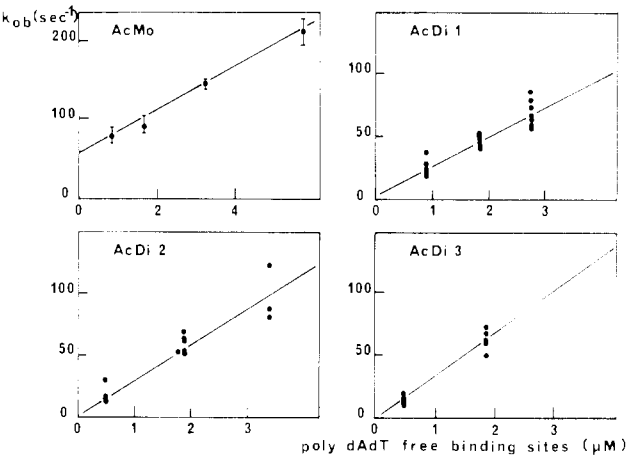



FIGURE 3: Pseudo-first-order analysis of acridine dimers and acridine monomer binding to poly[d(A-T)·d(A-T)]. The rate constants, $k_{obsd}$, are obtained from the reaction of acridine dimers with DNA in excess in an acetate buffer, 0.2 M, pH 5.0 at 20 °C. In each experiment, the ratio of free DNA/bound DNA at equilibrium is kept constant, with the assumption that the dyes are completely bound to DNA. Curves shown in this figure correspond to linear regressions of rate constants (weighted by standard errors) vs. DNA base pair molar concentrations. (AcDi 1) Free poly[d(A-T)·d(A-T)]/total dye, 42.5; $k_a = 23.4 \pm 2.0$ $\mu M^{-1}$ $s^{-1}$. (AcDi 2) Free poly[d(A-T)·d(A-T)]/total dye, 40.1; $k_a = 28.7 \pm 1.7$ $\mu M^{-1}$ $s^{-1}$. (AcDi 3) Free poly[d(A-T)·d(A-T)]/total dye, 48.2; $k_a = 34.5 \pm 4.5$ $\mu M^{-1}$ $s^{-1}$. (AcMo) Free poly[d(A-T)·d(A-T)]/total dye, 19.2; $k_a = 28.6 \pm 0.3$ $\mu M^{-1}$ $s^{-1}$; $k_d = 55.9 \pm 10$ $s^{-1}$.

Displacement of a dye:DNA complex (PX) by another DNA (Q) is normally accounted for by the following scheme

$$\begin{array}{ccc} P + X & \underset{k_d}{\overset{k_a}{\rightleftharpoons}} & PX \\ & + Q & \\ k_a' \downarrow & & \uparrow k_d' \\ & QX & \end{array}$$

In the case of the pseudo-first-order approximation, the free concentrations of each DNA does not differ significantly from the initial concentrations $P_0$ and $Q_0$. If the competing DNA, Q, is added to a preequilibrated mixture of P and X, the following equations apply

$$\frac{d(PX)}{dt} = k_a P_0(X) - k_d(PX) \quad (2)$$

$$\frac{d(X)}{dt} = k_d(PX) + k_d'(QX) - k_a' Q_0(X) - k_a P_0(X) \quad (3)$$

$$X_0 = (X) + (PX) + (QX) \quad (4)$$

where $X_0$ is the total concentration of the dye.

Combining eq 3 and 4:

$$d(X)/dt = (k_d - k_d')(PX) - (k_d' + k_a' Q_0 + k_a P_0)(X) + k_d' X_0 \quad (5)$$

The equation which describes the variations of (PX) with time is of the form:

$$(PX)_t - (PX)_\infty = A \exp(-k_{ex1} t) + B \exp(-k_{ex2} t) \quad (6)$$

$(PX)_t$ and $(PX)_\infty$ are the concentrations of PX at time $t$ and at equilibrium, respectively.

The two exchange rates, $k_{ex1}$ and $k_{ex2}$, are the roots of the equation

$$k_{ex}^2 - (k_d + k_d' + k_a P_0 + k_a' Q_0) k_{ex} + k_d k_d' + k_d k_a' Q_0 + k_d' k_a P_0 = 0 \quad (7)$$

with $\sum k = k_d + k_d' + k_a'Q_0 + k_aP_0$.

These roots are

$$k_{ex1,2} = \frac{\sum k}{2}\left(1 \pm \sqrt{1 - 4\frac{k_dk_d' + k_dk_a'Q_0 + k_d'k_aP_0}{(\sum k)^2}}\right) \quad (8)$$

In the case of acridine dimers, as $k_d$ and $k_d'$ are small, ($k_dk_d' + k_dk_a'Q_0 + k_d'k_aP_0$) is negligible compared with $(\sum k)^2$ and the first-order approximation holds:

$$k_{ex1,2} = \frac{\sum k}{2}\left(1 \pm \left(1 - 2\frac{k_dk_d' + k_dk_a'Q_0 + k_d'k_aP_0}{(\sum k)^2}\right)\right)$$

which gives the two approximated exchange rates:

$$k_{ex1} \simeq \sum k = k_d + k_d' + k_aP_0 + k_a'Q_0 \quad (9)$$

$$k_{ex2} \simeq \frac{k_dk_d' + k_dk_a'Q_0 + k_d'k_aP_0}{k_d + k_d' + k_a'Q_0 + k_aP_0} \quad (10)$$

When $Q_0$ is large, eq 10 simplifies and the exchange proceeds to a rate:

$$k_{ex2} \simeq k_d \quad (11)$$

$k_{ex1}$ corresponds to the first reequilibration of free dye by P and Q upon mixing. Its contributing amplitude can be evaluated since the concentration of the free dye, at time $t$, $(X)_t$, is described by an equation similar to eq 6:

$$(X)_t - (X)_\infty = C\exp(-k_{ex1}t) + D\exp(-k_{ex2}t) \quad (12)$$

The amplitudes of the first exponential on both sides of eq 2 can be identified:

$$-Ak_{ex1} = k_aP_0C - k_dA \quad (13)$$

taking $k_{ex1}$ from eq 9

$$A = -\frac{k_aP_0}{k_d' + k_a'Q_0 + k_aP_0}C \quad (14)$$

Therefore $A$, the amplitude of the fast relaxation, is smaller than $C$, and smaller than $X$ at $t = 0$ (eq 12), the concentration of free dye before any addition of the competing DNA. This free dye concentration is very small due to the high binding constant of the dye for DNA. Therefore, this fast relaxation in the case of acridine dimers has a very small amplitude far below our detection limits. In practice, a single relaxation rate is expected which should be equal to $k_d$.

In the case of the monomer, the approximations leading to eq 9 and 10 are still valid. Only the exponential corresponding to $k_{ex2}$ has a detectable amplitude. However, in that case, $k_d$ and $k_d'$ are much larger than in the case of the dimers; $k_{ex2}$ value is very close to that of $k_d$ only when the concentration of the competitor DNA (Q) is very large compared with (P). Thus when $Q_0$ becomes large, the relaxation rate tends to be equal to $k_d$ independent of the concentration of the competing DNA. The dissociation rate which is obtained in this exchange experiment (79 ± 8 $s^{-1}$) is reasonably close to the value deduced from pseudo-first-order analysis of the binding kinetics (56 ± 10 $s^{-1}$) (Figure 3).

Figure 4 shows an example of the relaxation observed after the addition of an excess of *Micrococcus luteus* DNA to a preequilibrated mixture of AcDi 1 and poly[d(A-T)·d(A-T)]. In the case of the acridine dimers AcDi 2 and AcDi 3, the exchange rates are independent of the concentration of the competing DNA (Table I). Thus the simple model proposed above is likely to apply. However the kinetics cannot be described by a single exponential. This probably indicates that

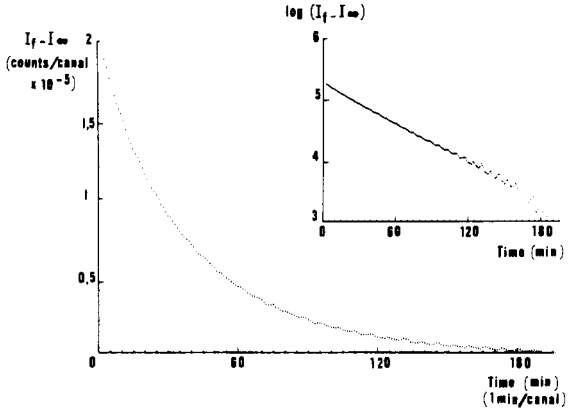



FIGURE 4: An example of a measurement of the exchange reaction between the AcDi 1–poly[d(A-T)·d(A-T)] complex and nonsonicated *Micrococcus luteus* DNA. The exchange is done from a solution containing 2.8 μM poly[d(A-T)·d(A-T)] preequilibrated with AcDi 1 ($r$ = 0.02) in acetate buffer, 0.2 M, pH 5.0 at 20 °C, to which is added *Micrococcus luteus* DNA to a final concentration of 100 μM. Fluorescence variation (arbitrary units) is plotted vs. time (1 min/canal) in a linear and a semilog representation. $I_F$ and $I_\infty$ represent fluorescence intensity at time $t$ and at infinite time, respectively.

Table I: Exchange Rate Constants for AcDi 2 in the Presence of Various Amounts of Displacing DNA[a]

| *M. luteus* DNA base pair concn (μM) | 23 | 47 | 200 | 280 | 400 | 520 |
|---|---|---|---|---|---|---|
| $k_{ex}$ for AcDi 2 ($s^{-1} \times 10^3$) | 1.22 | 1.07 | 1.00 | 1.07 | 1.68 | 1.27 |

[a] Measurements are carried out in 0.025 M acetate buffer, pH 5.0, with 2.8 μM poly[(d(A-T)·d(A-T)] at 20 °C ($r$ = 0.02). Values of exchange rate constants, $k_{ex}$, shown in this table, are the averages of the two observed relaxation rates weighted by their respective amplitudes (Schwarz, 1968).

Table II: Evolution of Relaxation Rate Amplitudes as a Function of Dye to DNA Ratio in the Case of AcDi 2[a]

| dye:DNA base pair ratio, $r$ | 0.02 | 0.14 | 0.19 | 0.30 | 0.40 |
|---|---|---|---|---|---|
| slower relaxation rate ($s^{-1}$) | 0.044 | 0.046 | 0.028 | 0.037 | 0.026 |
| % of total amplitude | 79 | 46 | 45 | 38 | 34 |
| faster relaxation rate ($s^{-1}$) | 0.18 | 0.17 | 0.14 | 0.16 | 0.15 |
| % of total amplitude | 21 | 54 | 55 | 62 | 66 |

[a] Measurements are carried out in 0.2 M acetate buffer, pH 5.0, with 0.8 μM poly[d(A-T)·d(A-T)] and 81 μM *Micrococcus luteus* DNA at 20 °C.

some heterogeneity occurs in the mode of binding. This explanation is supported by the evolution of the amplitudes of the two exponentials when the dye to phosphate ratio, $r$, is increased (Table II): the amplitude of the faster exponential increases at the expense of the slower one, while the corresponding rate constants are not significantly affected. Such behavior confirms the recent observation that, when $r$ ratio increases, secondary binding sites (where the dye covers two base pairs) will be favored with respect to primary binding sites (where the dye covers four base pairs; Gaugain et al., 1978).

The order of magnitude of the affinity constant, $K = k_a/k_d$ of the dimers for DNA, can be evaluated from the kinetic parameters for each composite relaxation. For this purpose, a mean dissociation rate constant, $k_d$, is defined, according to Schwarz (1968) as the arithmetical mean of the $k_d$ values weighted by their respective amplitudes. In 0.1 M acetate buffer, the binding constants of AcDi 2 and AcDi 3 for poly[d(A-T)·d(A-T)] ($2 \times 10^9$ and $1.5 \times 10^9$ $M^{-1}$, respectively) are found to be nearly a thousand times greater than the intrinsic binding constant of the monomer ($3 \times 10^6$ $M^{-1}$).

In the case of AcDi 1, the kinetics is found dependent on

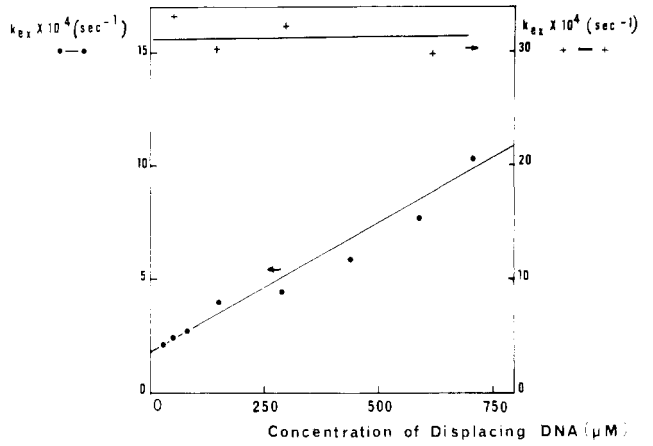



FIGURE 5: Dependence of the exchange relaxation rate ($k_{ex}$) of AcDi 1 as a function of the displacing DNA concentration. The observed mean relaxation rates are plotted vs. DNA concentration. (●—●) Displacing DNA, nonsonicated *Micrococcus luteus* DNA; poly[d-(A-T)·d(A-T)] concentration, 2.8 μM. Scale on the left. (+—+) Displacing DNA, sonicated *Micrococcus luteus* DNA; poly[d(A-T)·d(A-T)] concentration, 2.8 μM. Scale on the right. Measurements are done in sodium acetate buffer, 0.2 M, pH 5.0 at 25 °C. AcDi 1 concentration is 0.056 μM.

the DNA concentration (Figure 5) and the above dye exchange model is inadequate for the interpretation of the experimental data.

This phenomenon is indicative of the formation of a transient ternary complex between the dye and two DNA molecules as already discussed for other systems (Bresloff & Crothers, 1975; Blanquet et al., 1976; Giacomoni et al., 1977). As expected for bifunctional ligands, direct ligand transfer from one DNA molecule to the other can occur and it no longer requires dissociation of the ligand to the free state.

By using the same notation as above, exchange of the dye, X, between two DNAs, P and Q, can be schematized as shown in Scheme I.

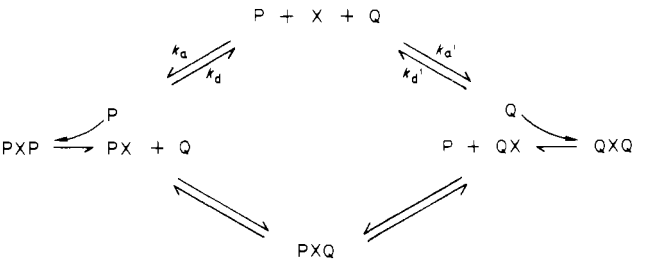
It should be noted that according to this scheme, a direct transfer will be significant only when the ternary PXQ complex can form at a rate faster than the rate of dissociation of the PX complex. Under this condition, in the case of the dye transfer from P to Q, direct transfer will depend on the relative stabilities of the PX and intermediate PXQ species. Two opposite situations can be met:

(a) The stability of the ternary complexes PXQ and PXP is such that their concentrations remain small compared with that of PX in the experimental conditions used. In that case, only PX and QX (the fluorescences of which are known) contribute to the measured fluorescence. The apparent rate of exchange increases simply with the competitor DNA concentration, tending toward $k_d$ as [Q] → 0.

(b) The stability of the PXQ and PXP species is significant. Their concentrations reach values of the order of the PX concentration. The results cannot be interpreted without prior determination of the fluorescence characteristics of the PXP, QXP, and QXQ species since these complexes contribute in large part to the observed fluorescence. In this situation, the observed rates of fluorescence variation could almost not be related to the rate of dissociation, $k_d$, of the PX complex.

From systematic experiments not presented here, it was inferred that the latter situation holds and that the following was probably occurring. Each of the PXP, PXQ, and QXQ species has a remarkably high stability even at the lowest DNA concentration as shown in Figure 5. Moreover the PXQ species has stability and fluorescence properties which depend on the species from which it originates, from the reaction of

Scheme I: Transfer through Free Ligand




"direct transfer"

PX with Q or from the reaction of QX with P. Actually it was observed that the strong fluorescence of the PX complex did not decrease upon reaction with an excess of Q toward PXQ. On the other hand, the weak fluorescence of QX did not increase upon reaction with P. In other words, this means that PXQ has fluorescence properties similar to that of the complex from which it originates. This behavior suggested that PXQ stabilization results from the interaction of the DNA competitor molecule with the polyamine interchain of the bisintercalated dye rather than from symmetrical sharing between the two DNAs of two acridine rings of the same dye. In the latter case, the fluorescence of the PXQ complex would have been quenched. This effect is to be related to the polyamine-induced aggregation of DNA (Osland & Kleppe 1977). It is expected that the stability of the PXQ complex will depend on the number of dyes involved in the DNA cross-bridging and, therefore, on the respective lengths of the reacting DNA molecules.

Indeed (Figure 5) after reducing the molecular weight of the competing DNA by sonication, the dependence of the rate of exchange on the DNA concentration is no longer observed. Moreover in that case, the observed rate of exchange becomes about ten times larger than the rate of exchange extrapolated at Q = 0 measured with the same DNA without prior sonication. Exchange experiments with high molecular weight DNA (T5 DNA mol wt = $76 \times 10^6$) were not feasible because immediate precipitation occurred even at the lowest tested concentrations. These results are in agreement with the model proposed above. It is therefore expected that the rate of exchange measured with sonicated DNA represents the true rate of dissociation ($k_d$) of the AcDi 1–poly[d(A-T)·d(A-T)] complex. The validity of this conclusion can be tested as follows: the rate of exchange and the corresponding association rate $k_a$ are used to calculate an apparent equilibrium constant $K_{ap}$ at different ionic strengths. The association rate of AcDi 1 to poly[d(A-T)·d(A-T)] is found roughly independent of ionic strength up to 0.5 M NaCl (Figure 6). In contrast, the exchange rate depends markedly on the ionic strength within any of the ranges explored. Beyond 0.5 M NaCl the equilibrium constant of AcDi 1 with poly[d(A-T)·d(A-T)] can be directly measured by fluorescence titration. Both sets of data fit the same linear relation and are plotted in Figure 7 on a log–log scale according to Daune (1972) and Record et al. (1976). This result supports our previous conclusion that the rate of exchange measured with sonicated DNA corresponds to the true dissociation rate $k_d$ of the AcDi 1–DNA complex. The binding constant in 0.1 M sodium acetate buffer, pH 5.0, of AcDi 1 for poly[d(A-T)·d(A-T)] may now be estimated. Its value, $2 \times 10^{11}$ $M^{-1}$, is more than 60 000 times larger than the corresponding constant with AcMo.

Similar estimations of the $k_d$ values for natural DNAs (AT-rich *Clostridium perfringens* DNA or GC-rich *Micrococcus luteus* DNA) were performed by reciprocal exchange by using sonicated DNAs. For both DNAs, the rate of ex-

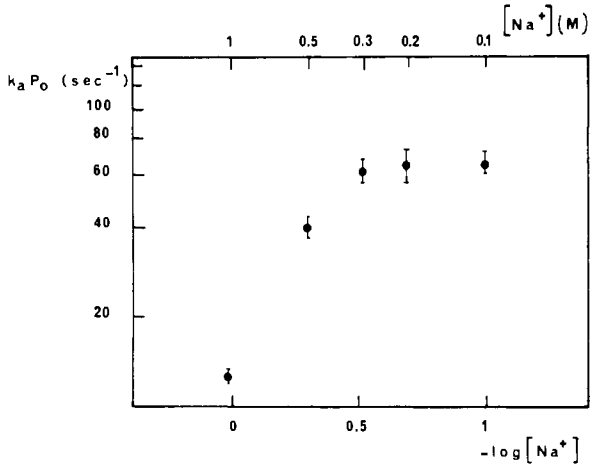



FIGURE 6: Effect of ionic strength on the rate of binding of AcDi 1 to poly[d(A-T)·d(A-T)]. A solution containing 2 × 1.86 μM poly[d(A-T)·d(A-T)] base pairs in 0.1 M sodium cacodylate (pH 6.0) plus increasing amounts of NaCl is mixed with an equal volume of solution containing 2 × 0.04 μM AcDi 1 in 1 mM sodium acetate (pH 5.0) and 0.1 M NaCl plus the same amounts of NaCl as in the poly[d(A-T)·d(A-T)] solution. Due to the negligible value of $k_d$, the observed rate of reaction is equivalent to the product of $k_a$ and of the concentration of poly[d(A-T)·d(A-T)] base pairs $P_0$ (eq 1).

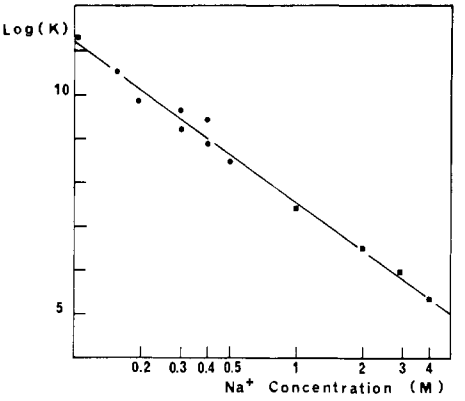



FIGURE 7: Variation of binding constants for AcDi 1 to poly[d(A-T)·d(A-T)] as a function of ionic strength. The full circles (●—●) correspond to binding constants evaluated from kinetic measurements at various ionic strength at pH 5.0, 20 °C (2.8 μM poly[d(A-T)·d(A-T)], $r$ = 0.02, and 47 μM sonicated *Micrococcus luteus* DNA). The full squares (■—■) correspond to binding constants directly measured at equilibrium by fluorescence titration.

change (in 0.2 M sodium acetate buffer, pH 5.0) is close to $1 \times 10^{-2}$ s$^{-1}$. If the association rate of AcDi 1 for these DNAs is identical with that measured in the case of poly[d(A-T)·d(A-T)], an apparent equilibrium constant of $2 \times 10^9$ M$^{-1}$ can be calculated. The value of the binding constant found for poly[d(A-T)·d(A-T)] in the same conditions (0.2 M sodium acetate buffer, pH 5.0) is $1 \times 10^{10}$ M$^{-1}$. It is significantly higher. A similar conclusion can be drawn by comparing these binding affinities of AcDi 1 for poly[d(A-T)·d(A-T)] and for *Micrococcus luteus* DNA obtained by equilibrium dialysis in a three-chambered dialysis cell (Table III).

The observed higher affinity of the dye to poly[d(A-T)·d(A-T)] than to other DNA could be related either to the special secondary structure of the synthetic polynucleotide or to a specific sequence effect. At the level of dinucleotides, it has been reported that several DNA intercalating dyes elicit a preference for the pyrimidine–purine sequence (Krugh & Reinhardt, 1975). With a pyrimidine–purine alternating copolymer, a dimeric dye such as AcDi 1 would be able to intercalate its two rings in the preferred sequences.

As shown in Figure 7, the plot of log $K$ vs. log ($Na^+$) for

Table III: Ratio of the AcDi 1 Binding Constant for Poly[d(A-T)·d(A-T)] to the AcDi 1 Binding Constant for *Micrococcus luteus* DNA Deduced from Equilibrium Dialysis[a]

| dye:DNA base pair ratio | 0.01 | 0.02 |
|---|---|---|
| AcDi 1 binding constant for poly[d(A-T)·d(A-T)]/AcDi 1 | 8.6 | 12.5 |
| | 11.1 | 14.0 |
| binding constant for *M. luteus* DNA | 10.1 | 13.9 |
| | (av: 9.9) | (av: 13.5) |

[a] Determinations are performed in a three-chambered cell as described in the Methods section in 0.2 M sodium acetate buffer, pH 5.0. The volumes and the concentrations of the solution of poly[d(A-T)·d(A-T)] in one chamber and *Micrococcus luteus* DNA in the other are identical (17 μM). The concentration of free dye being negligible, the ratio of the total dye concentrations measured in each chamber containing DNA equals the ratio of the corresponding DNA binding constants when the dye to DNA base ratio is small (Müller et al., 1973).

Table IV: Estimation of the Number of Interacting Charges[a]

| | | AcDi 1 | | | | |
|---|---|---|---|---|---|---|
| | AcMo | pH 5 | pH 6 | pH 7.4 | AcDi 2 | AcDi 3 |
| slope ($n\psi$) | 1.9 | 3.9 | 4.1 | 4.2 | 2.5 | 2.4 |
| calcd no. of interacting charges ($n$) | 2.2 | 4.4 | 4.7 | 4.8 | 2.8 | 2.7 |
| total no. of charge on the dye | 2 | 4 | 4 | 4 | 3 | 3 |

[a] The slopes are those of the best fits obtained in Figures 8 and 9 for log $K$ against log [$Na^+$]. The number of interacting charges is computed according to Record et al. (1976) and by using $\varphi$ = 0.88. The total number of charges corresponds to that of fully ionized dyes.

the AcDi 1–poly[d(A-T)·d(A-T)] interaction gives a straight line. According to Record et al. (1976), the slope of this line equals $n\psi$, $n$ being the number of interacting charges in the complex and $\psi$ a parameter equal to 0.88 for a double-stranded DNA. Similar behaviors are observed for AcMo, AcDi 2, and AcDi 3 binding to poly[d(A-T)·d(A-T)] (Figure 8). The comparison of the $n$ values with the corresponding number of charges of the dye is presented in Table IV.

(*4*) *Effect of pH.* The p$K$s of the dimers are two orders of magnitude lower than the p$K$ of the monomer (Le Pecq et al., 1975). This effect is attributed to the autostacking of the dimer rings. The dimer is in equilibrium between two forms (Barbet et al., 1976), the one open with the two acridine rings apart and the other folded with the two rings stacked. The latter conformation is predominant at high pH. Therefore, the kinetics of the binding is expected to vary when the pH increases because of the progressive deprotonation of the dye accompanied by the folding of the dye. In order to distinguish between the effect of the deprotonation and the conformational change of the dye, the following experiments have been carried out. The kinetics of dye binding have been recorded under two different conditions (Figure 9).

(i) The dye and DNA solutions in the same buffer of increasing pH are mixed in the stopped-flow apparatus.

(ii) The dye in a diluted acetate buffer solution (0.001 M, pH 5.0) and the DNA in a more concentrated buffer solution (0.2 M) of increasing pH are mixed. After mixing, the final pH does not differ from the pH of the DNA solution.

In the first type of experiment when the pH is above the p$K_a$ of the dye, the binding rate of the deprotonated folded dimer to DNA will be monitored.

In the second type of experiments, provided that the rate of dye folding is much slower than that of the DNA reaction, we can expect to monitor the binding of the deprotonated open dimer to DNA.

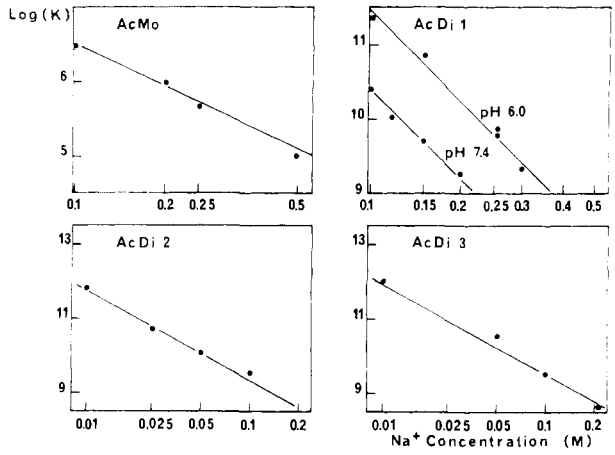



FIGURE 8: Compared variations of the binding constants of acridine monomer and dimers to poly[d(A-T)·d(A-T)] with ionic strength. For AcMo, the logarithm of intrinsic binding constants determined from titration curves at pH 5.0 (see Materials and Methods) is plotted vs. the logarithm of $Na^+$ concentrations. For AcDi 1, the logarithm of the ratio of $k_a$ and $k_d$, determined independently at pH 6.0 and pH 7.4, is plotted vs. the logarithm of $Na^+$ concentrations. For AcDi 2 and AcDi 3, the logarithm of the ratio of $k_a$ and $k_d$ at pH 5.0 is plotted vs. the logarithm of $Na^+$ concentrations ($k_a$ is assumed to remain constant in this range of ionic strength). All $k_d$ values are deduced from exchange measurements carried out with 2.8 $\mu$M poly[d(A-T)·d(A-T)] ($r = 0.02$) and 47 $\mu$M *Micrococcus luteus* DNA at 20 °C. Sonicated DNA is used in the case of AcDi 1.

In the former, a major slow process appears (Figure 9) which corresponds to a time constant of 30 s. The relative amplitude of this relaxation parallels the dye titration. In the latter, we observe that the binding rate constant remains unchanged up to pH 9. The corresponding measured time constant (18 ms) can be compared with the 30-s value obtained in the preceding experiment which was carried out with identical reactant concentrations and final pH. These results can be accounted for as shown in Scheme II.

While the binding rate constant, $k_a$, for the unfolded dye is independent of pH, the dissociation rate of the AcDi–poly[d(A-T)·d(A-T)] complex, $k_d$, as measured by the DNA exchange, decreases parallel to the deprotonation of the dye (results not shown). When the pH is increased and when it approaches the p$K$ value of the dimer, a dramatic fall in the binding affinity of AcDi 1 to DNA is observed. This results from the simultaneous occurrence of both the folding of the dimer and the destabilization of the dye–DNA complex.

## Discussion

Preliminary studies (Le Pecq et al., 1975; Gaugain et al., 1978) have shown that the equilibrium techniques were inadequate for the determination of the high binding constants of bifunctional intercalating drugs to DNA. This results from the difficulty of accurately measuring concentrations of free dye of the order of dissociation constants which are lower than $10^{-8}$ M. Alternatively, the binding constants can be estimated from the association and the dissociation rates of the equilibrium.

This study takes advantage of the enhancing effect of AT-rich DNA and of the quenching effect of GC-rich DNA on the fluorescence of acridines. In addition, the fluorescence-enhancing effect of acridine dimers is sequence dependent as these dimers are only fluorescent when bound to a sequence of four consecutive AT base pairs (Le Pecq et al., 1975). Because of this specific sequence effect on the fluorescence and possibly on the stability of acridines with DNA, most of the study has been performed with the homopolymer poly[d(A-T)·d(A-T)].

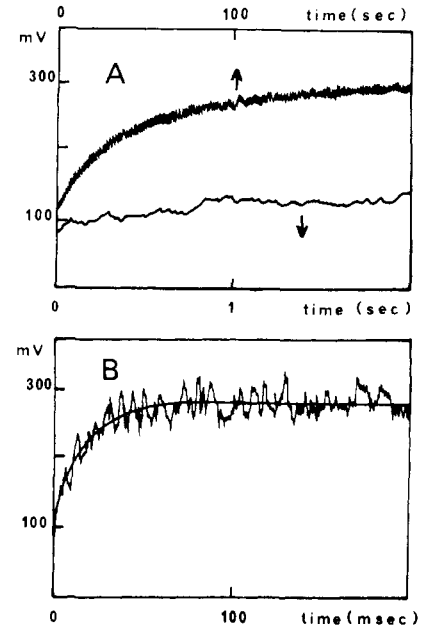
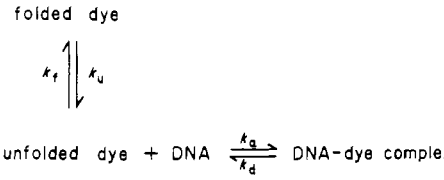



FIGURE 9: Relaxation for the formation of the poly[d(A-T)·d(A-T)]:AcDi 1 complex. (Panel A) A solution containing 2 × 1.86 $\mu$M DNA base pairs in 0.1 M Tris-HCl, pH 7.4, plus 0.1 M NaCl is mixed with an equal volume of a solution containing 2 × 0.043 $\mu$M AcDi 1 in the same buffer containing 0.1 M NaCl. (Panel B) A solution containing 2 × 1.86 $\mu$M DNA base pairs in 0.1 M Tris-HCl, pH 7.4, plus 0.1 M NaCl is mixed with an equal volume of a solution containing 2 × 0.043 $\mu$M AcDi 1 in 1 mM sodium acetate, pH 5.0, plus 0.2 M NaCl. The rise times are 10 ms (panel A) and 1 ms (panel B). The relaxations fit single exponentials with amplitudes of 0.178 ± 0.03 V (panel A) and 0.182 ± 0.017 V (panel B), and the rate constants are equal to 0.032 ± 0.002 $s^{-1}$ (panel A) and 57.6 ± 7.8 $s^{-1}$ (panel B). Relaxations of panel A correspond to the same experiments shown on two different time scales. The difference in time scale for the two panels should be noted.

Scheme II




Association rates of the order of $3 \times 10^7$ $M^{-1}$ $s^{-1}$ are found for acridine dimers binding to poly[d(A-T)·d(A-T)] and do not depend on the nature of the interchain of the various dimers under study. It is interesting to compare this value with that of the monomeric acridine ($k_a = 2.8 \times 10^7$ $M^{-1}$ $s^{-1}$). This result argues for identical modes of binding to DNA, possibly diffusion controlled. However, a value of the order of $10^8$ $M^{-1}$ $s^{-1}$ seems rather small for a simple diffusion-controlled process. A similar association rate constant value has been reported for irehdiamine binding to DNA (Dattagupta et al., 1978). In that case, two possibilities were considered:

(a) The ligand reacts only with a preexisting kink of DNA and the measured apparent association rate constant has to be weighted by the probability for opening a kink.

(b) A loosely electrostatistically bound irehdiamine molecule induces a kink at its site of binding. The second alternative was favored.

In the present case, the same two alternatives are encountered.

(a) Acridine rings can intercalate within preexisting sites such as kinks or small internal loops. The probabilities for kink formation and for opening a small internal loop in DNA

have been given by Sobell et al. (1977) and Gralla & Crothers (1973), respectively. Each are of the order of $10^{-3}$ per base pair. Interestingly enough, these values may depend on the local DNA sequence, the formation of a small internal loop being more frequent for an AT-rich sequence than for a GC-rich one (Gralla & Crothers, 1973). Under these conditions, an actual rate constant for the hypothetical reactions of diacridines and monoacridines with preexisting sites on DNA can be calculated. It is of the order of $10^{10}$ to $10^{11}$ $M^{-1}$ $s^{-1}$. According to this model which involves a conformational change of DNA prior to dye intercalation, two relaxations rates are expected. However, if the transient opening of DNA is in rapid preequilibrium compared with the dye binding process, a single relaxation will be observed.

(b) The conformational change of DNA follows dye–DNA encounter. Such a model has been considered in the case of proflavin binding to DNA of various AT and GC contents (Ramstein et al., 1972). In such a case, two relaxation rates are expected. However, depending on the fluorescence properties of the acridine in the various reaction steps, one of the relaxations could not be visualized.

In this work a single relaxation is observed for the binding process and distinction between the two alternative models is difficult. However, within the limits of accuracy of our experiments, the close correspondence between the equilibrium constants of AcMo for poly[d(A-T)·d(A-T)] as measured by prestationary or equilibrium techniques argues for no monomolecular rearrangement of the complex. A similar observation has been made in the case of ethidium bromide binding to DNA (Bresloff & Crothers, 1975). This tends to favor the model of dye binding to preexisting kinks or loops on DNA.

Because of the high stability of diacridines' complexes with DNA, dissociation rates had to be measured by exchange of the dye between DNAs of different base composition. Depending on $r$, the ratio of drug bound to DNA sites, several rates of exchange can be revealed in the cases of AcDi 2 and AcDi 3. This indicates heterogeneity in the binding of these dimers to DNA at high $r$ ratio. This is consistent with previous studies (Gaugain et al., 1978). At low $r$ values, the mean dissociation rate constants are measured allowing the calculation of the apparent equilibrium constants.

In the case of AcDi 1, a diacridine with a longer interchain, the interpretation of the exchange data is complicated by the appearance of a dependence on competing DNA concentration of the measured rate constants. This phenomenon which can be ascribed to the direct ligand transfer model of Bresloff & Crothers (1975) raises the interesting possibility that AcDi 1 can cross-bridge between DNA molecules. The interfering effect has been eliminated when sonicated DNA is used. Under these conditions, a rate constant of exchange is measured which no longer depends on the competing DNA concentration. From this value and the corresponding association rate constant, an apparent equilibrium constant of AcDi with poly[d(A-T)·d(A-T)] is estimated. If comparing the respective binding constants of AcMo, AcDi 1, AcDi 2, and AcDi 3, the following conclusions can be drawn. The dimerization of AcMo into any of the studied dimers leads to a marked increase in the binding constant to poly[d(A-T)·d(A-T)]. This is particularly striking in the case of AcDi 1 ($2 \times 10^{11}$ $M^{-1}$ at 0.1 M $Na^+$, pH 5.0) with respect to AcMo ($3 \times 10^6$ $M^{-1}$ in the same conditions). The expected gain into free energy of interaction by going from monomer to oligomer has been discussed in the case of the binding of oligosaccharides to lysozyme (Chipman & Sharon, 1969). Upon dimerization, the addition of the free energies of each subunit and of a (−2.4 kcal) term originating from the free energy of mixing represents the maximum free energy available for the binding of the dimer. The value found in the better case of AcDi 1 is about a thousand-fold smaller than the theoretical maximum. As usual, the loss in translational freedom and the geometric constraints upon binding to DNA may be evoked. AcDi 3 and AcDi 2, which differ only by the presence or absence of one methylene group in the interchain, are respectively a mono- and a bisintercalator of DNA (Le Pecq et al., 1975). Surprisingly, this does not lead to a major difference on their apparent binding constant to poly[d(A-T)·d(A-T)]. The on-rates of each diacridine are found to be identical, in agreement with the hypothesis of diffusion-limited binding. It remains to rationalize the fact that AcDi 2 and AcDi 3 dissociate at identical rates from DNA. The simplest explanation is that the side chains of each diacridine contribute differently to the stability of these complexes. It must be pointed out that the respective positions of nitrogens are different in the two dimers. Interestingly, it has been reported that thermine ($NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$), a natural polyamine from *Thermus thermophilus*, stabilizes nucleic acids significantly better than spermine ($NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) (Oshima, 1975). Therefore, the side chain of AcDi 3 which resembles the thermine structure might provide a larger amount of free energy for binding to DNA than the side chain of AcDi 2 which resembles the spermine structure. This difference may compensate for the lack of bisintercalation in the case of AcDi 3.

A striking result of this study is the finding that the reactivity of the dimer for DNA is dramatically affected by raising the pH. At pH 7.4, the association rate constant of AcDi 1 is reduced to 0.032 $s^{-1}$. It is shown that this slow rate corresponds to the limiting unstaking process of the dimer prior to its reaction with DNA. This result is in agreement with an earlier NMR study of diacridines (Barbet et al., 1976). The occurrence of self-stacked molecules at physiological pH clearly limits their reactivity with DNA and probably their biological and/or pharmacological activity. This observation prompted us to synthesize and study new dimers with more rigid interchains in order to prevent the folding of the dimers. This has led to the discovery of molecules with high antitumoral activities (unpublished results of these laboratories).

Another important factor for the biological activity of such molecules is their remarkably low rate of dissociation as discussed by Crothers (1971). It is noteworthy that the apparent rate of exchange of AcDi 1 between poly[d(A-T)·d(A-T)] and DNA decreases with the length of the DNA. The formation of a highly stable ternary complex of this dye with two DNA molecules slows down the exchange. The behavior could considerably affect the binding characteristics of such a derivative to DNA in the condensed phase occurring in vivo in the nucleus. Nevertheless, the affinity constant of AcDi 1 measured with sonicated DNA is already of the order of that *lac* repressor molecule for its operator DNA when measured in similar ionic strength conditions ($10^{11}$ $M^{-1}$ for repressor (Riggs et al., 1970) vs. $10^{10}$ $M^{-1}$ for AcDi 1 in 0.2 M salt).

The kinetical study of the binding of diacridines to DNA has allowed us to emphasize the importance of several factors on the mode of binding of DNA bisintercalators. Among these factors, the conformation and rigidity of the interchain have appeared as the most critical. The present results have been extremely useful for the design of new biologically active molecules in our laboratories.

References


Barbet, J., Roques, B. P., & Le Pecq, J. B. (1975) *C.R. Hebd. Seances Acad. Sci., Ser. D 281*, 851.

Barbet, J., Roques, B. P., Combrissons, S., & Le Pecq, J. B. (1976) *Biochemistry 15*, 2642.

Blanquet, S., Dessen, P., & Iwatsubo, M. (1976) *J. Mol. Biol. 103*, 765.

Bresloff, J. L., & Crothers, D. M. (1975) *J. Mol. Biol. 95*, 103.

Butour, J. L., Delain, E., Couland, D., Barbet, J., Roques, B. P. & Le Pecq, J. B. (1978) *Biopolymers 17*, 873.

Cain, B. F., Baguley, B. C., & Denny, W. A. (1978) *J. Med. Chem. 21*, 658.

Chen, T. K., Fico, R., & Canellakis, E. S. (1978) *J. Med. Chem. 21*, 868.

Chipman, D. M., & Sharon, N. (1969) *Science 165*, 454.

Crothers, D. M. (1968) *Biopolymers 6*, 575.

Crothers, D. M. (1971) *Prog. Mol. Subcell. Biol. 2*, 10.

Dattagupta, N., Hogan, M., & Crothers, D. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 4285.

Daune, M. P. (1972) *Eur. J. Biochem. 26*, 207.

Dervan, P. B., & Becker, M. M. (1978) *J. Am. Chem. Soc. 100*, 1968.

Di Marco, A., Casazza, A. M., Gambetta, R., Supino, R., & Zunino, F. (1976) *Cancer Res. 36*, 1962.

Gaugain, B., Barbet, J., Capelle, N., Roques, B. P., & Le Pecq, J. B. (1978) *Biochemistry 17*, 5078.

Giacomoni, P. U., Delain, E., & Le Pecq, J. B. (1977) *Eur. J. Biochem. 78*, 215.

Gralla, J., & Crothers, D. M. (1973) *J. Mol. Biol. 78*, 301.

Jain, S. C., Tsai, C. C., & Sobell, H. M. (1977) *J. Mol. Biol. 114*, 317.

Jovin, T. M. (1976) *Annu. Rev. Biochem. 45*, 889.

Krugh, T. R., & Reinhardt, C. G. (1975) *J. Mol. Biol. 97*, 133.

Kuhlmann, K. F., Charbeneau, N. J., & Mosher, C. (1978) *Nucleic Acids Res. 5*, 2629.

Le Pecq, J. B., & Paoletti, C. (1967) *J. Mol. Biol. 27*, 87.

Le Pecq, J. B., Nguyen-Dat-Xuong, Gosse, C., & Paoletti, C. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 5078.

Le Pecq, J. B., Le Bret, M., Barbet, J., & Roques, B. P. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 2915.

Lerman, L. S. (1961) *J. Mol. Biol. 3*, 18.

Li, H. J., and Crothers, D. M. (1969) *J. Mol. Biol. 39*, 461.

Mc Ghee, J. P., & Von Hippel, P. M. (1974) *J. Mol. Biol. 86*, 469.

Müller, W., & Crothers, D. M. (1968) *J. Mol. Biol. 35*, 251.

Müller, W., Crothers, D. M., & Waring, M. J. (1973) *Eur. J. Biochem. 39*, 223.

Oshima, T. (1975) *Fed. Eur. Biochem. Soc., Meet., 10th*, 1423.

Osland, A., & Kleppe, K. (1977) *Nucleic Acids Res. 4*, 685.

Ramstein, J., Dourlent, M., & Leng, M. (1972) *Biochem. Biophys. Res. Commun. 47*, 874.

Record, M. T., Jr., Lohman, T. M., & De Haseth, P. (1976) *J. Mol. Biol. 107*, 145.

Riggs, A. D., Suzuki, H., & Bourgeois, S. (1970) *J. Mol. Biol. 48*, 67.

Roques, B. P., Barbet, J., Oberlin, R., & Le Pecq, J. B. (1976a) *C.R. Hebd. Seances Acad. Sci., Ser. D 283*, 1365.

Roques, B. P., Barbet, J., & Le Pecq, J. B. (1976b) *C.R. Hebd. Seances Acad. Sci., Ser. D 283*, 1453.

Sakore, T. D., Jain, S. C., Tsai, C. C., & Sobell, H. M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 188.

Schwarz, G. (1968) *Rev. Mod. Phys. 40*, 206.

Sobell, H. M., Jain, S. C., Tsai, C., & Gilbert, S. G. (1977) *J. Mol. Biol. 114*, 333.

Tsai, C. C., Jain, S. C., & Sobell, H. M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 628.

Von Hippel, P. H., Revzin, A., Cross, C. A., & Wang, A. C. (1975) in *Protein–Ligand Interactions* (Sund, H., & Glauer, G., Eds.) pp 270–288, de Gruyter, Berlin.