*Proc. Nat. Acad. Sci. USA*
Vol. 72, No. 8, pp. 2915–2919, August 1975
Biochemistry

# DNA polyintercalating drugs: DNA binding of diacridine derivatives

(DNA intercalation/DNA unwinding/acridine/spermidine/spermine)

JEAN-BERNARD LE PECQ*, MARC LE BRET*, JACQUES BARBET†, AND BERNARD ROQUES†

* Laboratoire de Pharmacologie Moléculaire associé au CNRS no. 147, Institut Gustave-Roussy 94800 Villejuif, France; and † Département de Chimie, Ecole Polytechnique, 17, rue Descartes—75230 Paris Cedex 05, France

*Communicated by Norman Davidson, May 7, 1975*

**ABSTRACT** **As a first step in the synthesis and the study of DNA polyintercalating drugs, dimers of acridines have been prepared. Their DNA binding properties have been studied. It has been determined that when the chain separating the two aromatic rings is longer than a critical distance, bisintercalation is actually observed and that the DNA binding affinity becomes quite large ($>10^8$–$10^9$ $M^{-1}$). It is shown also that the optical characteristics of these molecules are dependent on the sequences of DNA. The fluorescence intensity of one of these dimers when bound to DNA varies as the fourth power of its A+T content. This derivative could be used as a fluorescent probe of DNA sequence.**

A large variety of drugs are known to interact strongly with nucleic acids. Many bind specifically to double-stranded nucleic acids through intercalation between adjacent base pairs, as first described by Lerman (1). To this class of compounds belong several antitumoral agents of clinical importance (actinomycin D, adriamycin, and daunomycin) as well as several drugs used in parasitic diseases (ethidium bromide, quinacrine, chloroquine, miracil D, etc.). These biological properties very probably result from the binding of the drugs to DNA. It is therefore of interest to try to design molecules that have the highest possible affinity for DNA when biological and pharmacological properties are sought. Such an attempt led us recently to a new antitumoral agent in the series of ellipticines (2, 3). However, the DNA binding affinity of all these compounds to polynucleotides remains weak ($10^5$–$10^7$ $M^{-1}$) when compared to the DNA affinity of the DNA-dependent RNA polymerase and of the repressor ($10^{10}$–$10^{13}$ $M^{-1}$) (4–6). It is therefore logical, in a search for compounds able to interfere with the biological functions of replication and transcription, to try to obtain molecules with DNA affinity of the same order of magnitude. As a consequence we began a research program following the principle that such affinities can be reached using polymers of intercalating molecules. The binding free energy of each subunit will add up provided that entropic factors are not too unfavorable and the resulting binding affinity will increase rapidly with the number of subunits. To test the feasibility of such an idea, acridine dimers of which the two aromatic rings are linked by chains of various lengths and structures have been prepared‡. In this paper we report the main DNA binding characteristics of these compounds.

## MATERIALS AND METHODS

The structure of the derivatives that have been studied here is shown in Fig. 1. They have been synthetized according to procedures that have been described earlier‡.

‡ J. Barbet, B. P. Roques, and J. B. Le Pecq, *Comptes Rendus serie D*, in press.

Calf thymus DNA was prepared in the laboratory according to Aubin *et al.* (7). T7 and λ phage DNAs were prepared from the purified phage solutions by three phenol extractions. DNAs of yeast mitochondria, crab poly[d(A-T)], *Proteus mirabilis*, phage T5, and herpes virus were gifts, respectively, from Drs. Guerineau, Bernardi, Festy, Jacquemin-Sablon, and Sheldrick. DNAs from *Clostridium perfringens* and *Micrococcus luteus* were purchased from Sigma and further purified by phenol extraction.

Bacteriophage PM2 (strain from Dr. Espejo) and PM2 DNA, a covalently closed circular DNA (8), were prepared in the laboratory as described (8, 9).

Alternating poly[d(A-T)] and the *Neurospora crassa* endonuclease (EC 3.1.4.-) were obtained from Boehringer.

Calf thymus sonicated DNA was prepared as described (10). In addition, to remove an eventual denatured DNA fraction, the sample was treated by the *Neurospora crassa* endonuclease [0.5 unit/ml as defined by Linn and Lehman (11)] for 25 min at 45°. In a parallel control experiment, denatured DNA treated under the same conditions became completely acid-soluble. Sonicated DNA was then extracted with phenol to remove the nuclease. Measurement of the sedimentation coefficient (7 S) indicated that the molecular weight of the DNA obtained after such a treatment was of the order of $3 \times 10^5$, according to the relation of Eigner and Doty (12) valid for low-molecular-weight DNA.

Fluorescence measurements were done with a photon counting spectrofluorometer built in this laboratory (13) at 20°.

Fluorescence decay times have been measured by the time correlated single photon counting technique (reviewed in ref. 14) with an instrument built in this laboratory.

The unwinding angle of the DNA helix caused by the binding of the different derivatives was measured with covalently-closed circular DNA from PM2 phage using viscosimetry (15).

To measure the length increase of short DNA segments, the intrinsic viscosity of sonicated DNA in the presence of

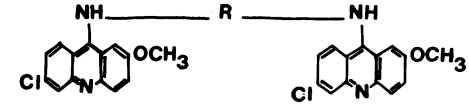


FIG. 1. Diacridine structure. The properties of the diacridines are compared to a monoacridine derivative that has the same acridine ring as the dimers shown in this figure and the chain —NH—$(CH_2)_3$—N—$(CH_3)_2$ attached to position 9 of the acridine ring. This monomeric molecule [2-methoxy-6-chloro-9-(3-dimethyl amino propylamino) acridine] is referred in this paper as *compound I. Compound IIA:* same structure as shown in the figure with R = —$(CH_2)_3$—NH—$(CH_2)_4$—NH—$(CH_2)_3$—. *Compound IIB:* R = —$(CH_2)_3$—NH—$(CH_2)_4$—. *Compound III:* R = —$(CH_2)_3$—NH—$(CH_2)_3$—.

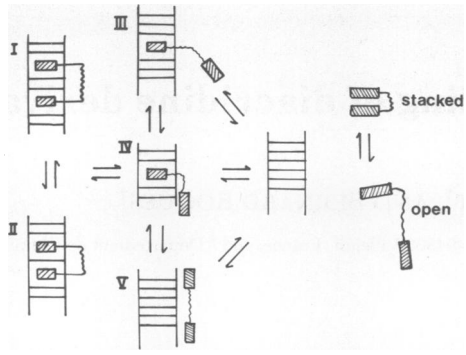


FIG. 2. Schematic representation of the different possible binding mechanisms of the dimeric molecules to DNA.

increasing concentration of the various derivatives was measured. log $[\eta]/[\eta]_0$ was plotted as a function of log $(1 + 2r)$ according to Saucier *et al.* (10), $[\eta]$ and $[\eta]_0$ being, respectively, the intrinsic viscosities of the DNA measured in the presence and in the absence of increasing concentrations of the acridine dyes, and $r$ being the number of bound dye molecules per nucleotide on DNA. As previously shown (10), the slope of such curve can vary between 2 and 3 for the intercalation of a monomer, and is expected to be between 4 and 6 in the case of a bisfunctional intercalating derivative.

## RESULTS

Among the different molecules that we have prepared we have chosen to study particularly the molecules shown in Fig. 1. Some of these molecules are symmetrical (IIA and III). The chain linking the two acridine rings is identical to spermidine (IIB) or spermine (IIA). These natural polyamines have been chosen because they are known to bind well to DNA and therefore it was thought that their structures were adapted to fit in the groove of the DNA helix. X-ray diffraction structures of these derivatives (C. Courseille, B. Busetta, J. Barbet, and M. Hospital, *Acta Crystallogr.*, submitted) are in agreement with this point of view. Owing to the interaction between the two rings, which is seen in nuclear magnetic resonance studies (J. Barbet, B. P. Roques, S. Combrisson, and J. B. LePecq, *Biochemistry*, submitted), the $pK_a$s of the acridine rings of the dimers ($pK_{aIII}$ = 6.5) are several orders of magnitude lower than the monomer $pK_a$ ($pK_{aI}$ = 8.5). All the measurements have therefore been performed at pH = 5.0, where the acridine rings of all the derivatives are fully ionized.

The acridine dimers can possibly bind to DNA according to different models that we have schematized in Fig. 2, depending on whether one or two of the aromatic rings are bound to the outside of the DNA helix or intercalated. In order to investigate whether the two rings were really intercalated and what was the influence of the chain length, we measured the unwinding angle of the DNA helix using supercoiled DNA and the length increase of short DNA segments caused by the binding of the monomer compared to that of the different dimers. The results of the measurements are presented in Figs. 3 and 4. These experiments show that compounds IIA and IIB unwind and increase the length of the DNA helix twice as much as the monomer does. In contrast, compound III, which has the shortest chain, unwinds the DNA helix by the same amount as the monomer. The increase of viscosity of the short DNA segments observed for compound III is larger than the monomer, but the slope of the curve of log $[\eta]/[\eta]_0$ against log $(1 + 2r)$ (Fig. 4) is less than double that of the monomer. Its value is compatible only with a monointercalation. Its higher value is probably the result of a rigidification of the DNA helix caused by the binding of the second aromatic ring on the outside of the DNA helix.

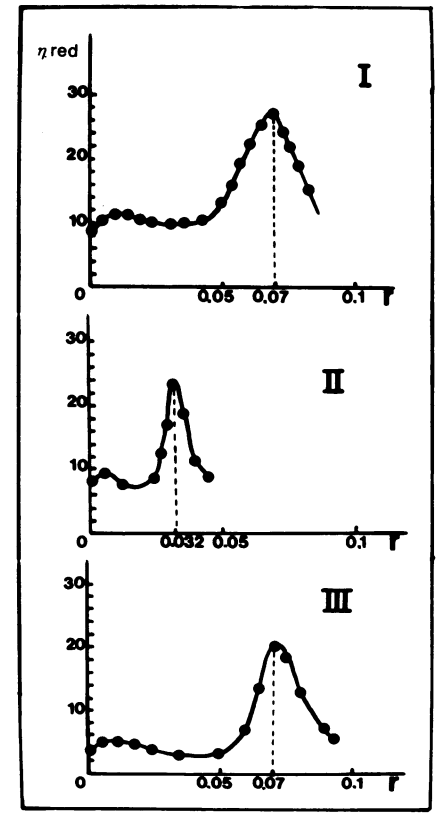


FIG. 3. Determination of the unwinding angle of the DNA helix caused by acridine dimers. The reduced viscosity is measured as a function of $r$ (number of dye molecules bound per nucleotide). The DNA concentration used is 150 $\mu$g/ml in 0.2 M acetate buffer (pH 5.0). Parts I, II, and III of the figure are the results obtained, respectively, for compounds I, IIB, and III. The result obtained with compound IIA is not shown, but is identical to that of IIB. The maximum of the viscosity corresponds to the relaxed circle. The value of $r$ at this point ($r_e$) is inversely proportional to the unwinding angle of the DNA helix caused by the added dye. For ethidium bromide, $r_e$ is, under the same conditions, found equal to 0.047 (10). Because the DNA unwinding angle of ethidium is 26° (22), the unwinding angle caused by compounds I, IIA, IIB, and III are therefore, respectively, 17°, 38°, 38°, and 17°. The DNA unwinding angle found for the monomeric compound (I) is similar to that of other related acridine derivatives (10).

In order to determine the DNA binding affinity and the stoichiometry of the reaction, we have measured the fluorescence increment of the solution in the presence of poly[d(A-T)] for increasing concentrations of the dyes. Because, as it will be shown later, the fluorometric characteristics of the derivatives are dependent on the sequence of the DNA, the use of a homopolymer simplifies the interpretation. Furthermore, this methodology is convenient for making the measurements at very low concentrations, which are necessary when the affinities are large. The results are shown in Fig. 5. The apparent complexity of the results is in relation to the multiplicity of the different forms in the bound state (Fig. 2). For the three compounds, when the value of the ratio dye/DNA-P is below 0.1, the fluorescent titration curve is linear and one can compute the amount of bound dye in the

same way as Le Pecq and Paoletti (16) because there is either no change (compound IIA) of the quantum yield of the fluorescence of the bound dye or a small change which can be determined from the measurements of fluorescence lifetime. In all cases we find that 100% of the dye is bound. When the ratio dye/DNA-P is above 0.1, the calculation can still be done for compound IIA since the fluorescence lifetime does not change. We find that in this case 100% of the dye is bound until the dye/DNA-P ratio reaches 0.2, after which no more binding is observed. For compounds IIB and III, the curvature of the titration curves observed after dye/DNA-P > 0.1 results almost completely from a change of the quantum yield of fluorescence of the bound dye, as demonstrated by the parallel change of the decay time of fluorescence. We conclude, therefore, that in the three cases the stoichiometry of the binding is 1 dye molecule per 5 nucleotides and that no dissociation of the complex is observed. Therefore no binding constant can be determined. Nevertheless a minimum binding constant can be computed. If the Scatchard equation $r/c = Kn - Kr$ (with $r$ being the number of dye molecules bound per nucleotide, $c$ the free dye concentration, $n$ the number of sites per nucleotide, and $K$ the association constant) is expressed as a function of $D_t$ (the total dye concentration) and $P_t$ (the total DNA concentration expressed in nucleotides), we get:

$$r^2P_t - r(P_tn + D_t + K^{-1}) + nD_t = 0 \qquad [1]$$

of which root is:

$$r = \frac{(nP_t + D_t + K^{-1}) - \sqrt{(P_tn + D_t + K^{-1})^2 - 4nD_tP_t}}{2P_t} \qquad [2]$$

Let us assume that the saturation of a given series of sites corresponds to $n = 0.1$. Then, when $nP_t = D_t$, one can calculate from Eq. [2] that if $D_t = K^{-1}$ the fraction of occupied sites on DNA is $r/n = 0.38$, for $D_t = K^{-1} \times 10$, $r/n = 0.73$, and for $D_t = K^{-1} \times 100$, $r/n = 0.90$.

Because we cannot measure even 10% of free dye, it can be concluded that $K^{-1} < D_t/100$, and from the data of Fig. 5, $K^{-1} < 1.8 \times 10^{-9}$ M.

Under the same conditions the value of $K^{-1}$ for the monomer is found equal to $0.5 \times 10^{-5}$ M. This means that the binding affinity in this range is at least 2000 to 3000 times higher for the dimer than for the monomer. In order to measure such a high binding constant it would be necessary to perform the measurements at a concentration of the order of $10^{-9}$ M. That would be possible in theory using fluorescence and photon counting. Attempts to make such measurements failed because at these low concentrations the adsorption of the dyes to the glass becomes a difficult problem to solve. From the viscosity data of Figs. 3 and 4 and from the fluorimetric titration of Fig. 5 it can be concluded:

(*i*) for compound III, only one of the two acridine rings is intercalated, and the interaction of the second ring with the outside of the DNA helix is responsible for the increase of the DNA affinity. The stoichiometry of the binding corresponds to $n$ close to 0.2.

(*ii*) For compounds IIA and IIB, below $r = 0.125$, which represents the supposed intercalation limit for a dimer since the limit for a monomer is 0.25., the two rings are intercalated. This is clearly shown by the facts that the unwinding angle, the length increase of the DNA helix, is double that observed for the monomer. Above $r = 0.125$ it is not clear what happens. No valuable information can be obtained from the change of viscosity of the short DNA segments (Fig. 4) because the drop of viscosity after $r = 0.13$ is later on accompanied by a precipitation only observed here because of the high DNA concentration necessary to perform this experiment. Nevertheless from experiments now in progress (measurement of DNA length by electron microscopy) we believe that in this range only one of the two rings of the dimer is intercalated since the other becomes bound on the outside of the helix (equilibrium between forms I, II, and IV of Fig. 2). This would explain the observed stoichiometry of the reaction ($n = 0.2$), which corresponds to a monointercalation. This complex behavior probably results from the small difference in the free energy of binding between the intercalated site and the outside binding which is classical with the acridine derivatives.

It follows from these results that the properties of the dimers when bound to DNA could be dependent on the sequence of this DNA. Indeed we have observed such a characteristic. The fluorescence quantum yield of the monomer is high when intercalated in poly[d(A-T)] and very low on poly[d(G-C)] as for quinacrine (17), which is closely related. It is, therefore likely, that when such an acridine ring is intercalated at the contact of a G-C pair, its fluorescence is quenched. In the dimer, owing to resonance energy transfer, if only one of the two rings is at the contact of a G-C pair the fluorescence will be quenched. Therefore, it is expected that several adjacent A-T base pairs would be necessary to get no quenching of fluorescence. This is indeed observed, as shown in Fig. 6. The fluorescence intensity of the dimer IIA bound to DNAs of various A-T content varies as the fourth power of the A-T fraction, as if four consecutive pairs were constituting the binding site. The fluorescence of this compound can therefore probe the sequence of the DNA and can easily differentiate between synthetic poly[d(A-T)] and crab poly[d(A-T)]. It must be pointed out that the binding itself is independent of sequence, as shown by the measurement of the fluorescence on a mixture of DNAs of different base composition.

## DISCUSSION

It is clear from the viscosimetric determinations that in the range of these measurements ($r < 0.1$) the two dimers with the longest chains (IIA and IIB) bisintercalate and that only one of the two rings of the dimer with the shortest chain is intercalated. It is also clear from these experiments that the bisintercalation occurs on the same DNA molecule and does not result in a crosslinking of different DNA molecules. This conclusion is also in agreement with experiments not shown here (electron microscopy and sedimentation behavior of DNA in the presence of these dyes). An interesting question to ask is whether these two dimeric molecules when bisintercalated are bound according to model I or II of Fig. 2, leaving between their aromatic rings one or two base pairs. The so called "excluded site" model (18, 19), which proposes that two intercalated molecules have to be separated by at least two base pairs, would tend to support model one. Therefore,

it is interesting to compare the length of the chain of the three dimers studied here to the minimal length needed to join two intercalated molecules in models I and II.

(*i*) *Model I.* A minimal length for the chain is of course the length along the DNA axis, 10.1 Å. The actual length needed to join the two subunits is certainly longer and depends on the angle between the two rings and on the position of the axis of rotation of the dimer, which could well be different from the DNA axis.

(*ii*) *Model II.* In this case because the rotation between the two rings is small, the minimal distance is close to the length separating the two rings along the DNA axis, 6.7 Å.

These two values can now be compared to the maximum length of the chain linking the acridine rings of the various dimers. These lengths can be computed (with C-C and C-N length being, respectively, 1.53 and 1.47 Å and the nitrogen in the middle of the chain protonated) or derived from model building data. We found, respectively, for these compounds: IIA, 16.1 Å; IIB, 11.2 Å; and III, 9.9 Å. The length of these chains is always compatible with model II, and according to this model we would expect that the three dimers could bisintercalate as well.

If model I is exclusive, compound III, which has the shortest chain, would not bisintercalate, in agreement with experiment. The compound with the longest chain would bisintercalate with no difficulty. The case of the compound with the chain of intermediate size is more difficult to decide. The length of the chain is critical, and even if it is difficult to build a model in agreement with model I, it is not possible to rule it out completely. Additional evidence on the relative positions of the chromophores are needed to conclude safely, but one cannot but be stricken by the fact that the removal of a single carbon is able to suppress the bisintercalation, as expected from model I.

The potential uses of such molecules are numerous. We would like to comment on a few of them. It could be possi-

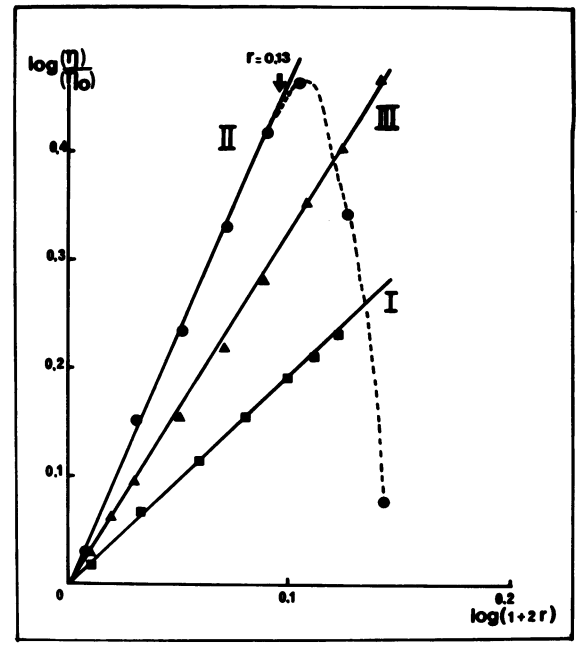


FIG. 4. Determination of the length increase of sonicated DNA caused by monomeric and dimeric acridines. Intrinsic viscosities of sonicated DNA are measured in the absence of dye ($[\eta]_0$) and in the presence of increasing concentration of dye $[\eta]$. $r$ is the number of dye molecules bound per nucleotide. DNA concentration is 150 μg/ml in 0.2 M acetate buffer (pH 5.0). *Curve I* (■) is the result obtained with the monomeric derivative: *compound I* of Fig. 1. *Curve II* (●) refers to the dimeric compound IIA. For simplicity, the result of compound IIB is not shown and is almost identical to the result shown for IIA. *Curve III* (▲) refers to dimeric compound III (shortest chain). For curve II, a rapid decrease of viscosity is observed after $r = 0.13$, which is later accompanied by a precipitation of the DNA-dye complex.

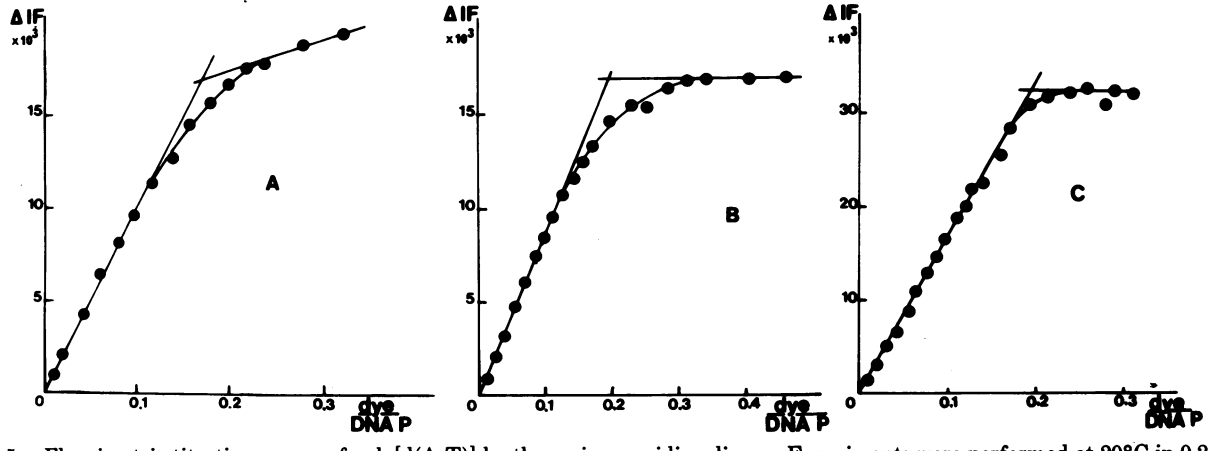


FIG. 5. Fluorimetric titration curves of poly[d(A-T)] by the various acridine dimers. Experiments were performed at 20°C in 0.2 M acetate buffer (pH 5.0). Concentration of poly[d(A-T)] is 0.55 μg/ml in the three cases. The fluorescent increment, ΔIF, is the difference of fluorescence intensity between the solution containing the dye and the poly[d(A-T)] and the solution containing the dye alone at the same concentration and measured under identical conditions. It is expressed as the number of impulses counted per 10 sec in the photon counting instrument and is the average of three determinations. The ratio dye/DNA-P is the ratio of the molar total dye concentration over the molar concentration of poly[d(A-T)] expressed in nucleotides. Curves A, B, and C refer, respectively, to the results obtained with compound III (short chain dimer), IIB (medium size chain dimer), and IIA (long chain dimer). At this DNA concentration, the fluorescence increase and, therefore, the binding of the monomeric acridine derivative (III) is too small to be measured accurately. At higher DNA concentration, the binding constant is easily measured and found equal to $2.0 \times 10^5\ M^{-1}$. To check the constancy of the quantum yield of fluorescence of the bound dye during the titration, fluorescence lifetimes have been measured. For compound IIA (longest chain; part C of this figure), the fluorescence lifetime was constant over the entire titration curve and equal to 28.4 nsec. For compound IIB (part B of this figure), the lifetimes were, respectively, for $r = 0.05$, $r = 0.1$, $r = 0.15$, and $r = 0.2$, 18.2 nsec, 17.1 nsec, 16 nsec, and 15.2 nsec. For compound III (part A of this figure), the fluorescence lifetimes were, respectively, for $r = 0.05$, $r = 0.1$, $r = 0.15$, and $r = 0.2$, 25 nsec, 23 nsec, 20.1 nsec, and 18.7 nsec. The fluorescence decays of the free dyes are made up of two exponentials and correspond probably to the open and stacked forms of the dimers.

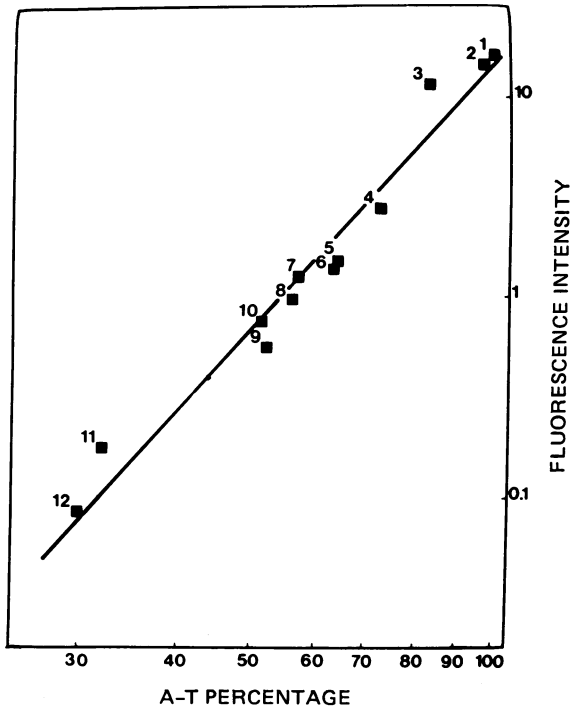


FIG. 6. Variation of the fluorescence intensity of the longest chain dimer (IIA) when bound to DNA as a function of their A-T percentage (log-log plot). Measurements are made in 0.2 M acetate buffer (pH 5.0). Excitation wavelength is 460 nm; emission wavelength 495 nm; the fluorescence intensity obtained for a given DNA is measured relative to calf thymus DNA, which is taken as one. The DNA concentration expressed in nucleotides was $2 \times 10^{-5}$ M, and the dye concentration was $2 \times 10^{-7}$ M. The different DNAs used were: *1*, synthetic poly[d(A-T)]; *2*, crab poly[d(A-T)]; *3*, yeast mitochondrial DNA; *4*, *Clostridium perfringens* DNA; *5*, *Proteus mirabilis* DNA; *6*, T5 DNA; *7*, PM2 DNA (nicked); *8*, calf thymus DNA; *9*, T7 DNA; *10*, λ DNA; *11*, herpes virus DNA; *12*, *Micrococcus luteus* DNA.

ble to build molecules able to recognize double helices with the A structure by fixing the angle between the chain and the aromatic rings. The possibility to get sequence specific molecules is of course of great interest, especially with respect to the chemotherapy of viral and cancerous diseases. A trimer would not be too difficult to build. It would certainly have a very high DNA affinity. Its properties could also be a little different from that of the dimer. By going possibly through states I, II, III, IV, and V shown on Fig. 2, the dimer can creep along the DNA helix. The trimer could not do that so easily, and its binding would look more like the binding of a repressor.

Polyintercalating drugs will unwind the DNA helix much more than ordinary intercalating dyes. Because the binding affinity to supercoiled DNA is related to the size of this unwinding (20), the specificity of these molecules for supercoiled DNA could become high.

Much progress has been made recently in the isolation of some pharmacological receptors. It is possible that some of them are made of dimeric or oligomeric proteins. It seems to us that it would be therefore of interest to study the properties of dimeric molecules made up of pharmacologically active compounds separated by chains of various length. Recently a new antibiotic, echynomycin, has been shown to bisintercalate in DNA (21). This observation confirms the usefulness of this approach.

The technical assistance of Mrs. J. Couprie has been very much appreciated. This work was supported by the CNRS (ATP Pharmacologie) la Fondation pour la Recherche Médicale, l'Institut Gustave-Roussy (contrat de Recherche Clinique), and l'Université Pierre et Marie Curie (Paris VI). Preliminary experiments were performed with an acridine dimer synthetized by Dr. Kraayenhoff, whose advice was of great help for the synthesis of new derivatives.

1. Lerman, L. S. (1961) *J. Mol. Biol.* **3**, 18–30.
2. Le Pecq, J. B., Le Bret, M., Gosse, Ch., Paoletti, C., Chalvet, O. & Nguyen-Dat-Xuong (1974) "Jerusalem symposia on quantum chemistry and biochemistry," in *Molecular and Quantum Pharmacology*, eds. Bergman, E. D. & Pullman, B. (D. Reidel, Dordrecht-Holland), Vol. 7, pp. 501–521.
3. Le Pecq, J. B., Nguyen-Dat-Xuong, Gosse, C. & Paoletti, C. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 5078–5082.
4. Hinkle, D. C. & Chamberlin, M. J. (1972) *J. Mol. Biol.* **70**, 157–185.
5. Riggs, A. D., Suzuki, H. & Bourgeois, S. (1970) *J. Mol. Biol.* **48**, 67–83.
6. Von Hippel, P. H. & Mc Ghee, J. D. (1972) *Annu. Rev. Biochem.* **41**, 231–300.
7. Aubin, G., Chenaille, P., Lamonthezie, N. & Paoletti, C. (1963) *Biochim. Biophys. Acta* **72**, 456–468.
8. Espejo, R. T., Canelo, E. S. & Sinsheimer, R. L. (1969) *Proc. Nat. Acad. Sci. USA* **63**, 1164–1168.
9. Le Pecq, J. B. (1971) in *Methods of Biochemical Analysis*, ed. Glick, D. (J. Wiley & Sons, New York), Vol. 20, pp. 41–86.
10. Saucier, J. M., Festy, B. & Le Pecq, J. B. (1971) *Biochimie* **53**, 973–980.
11. Linn, S. & Lehman, I. R. (1965) *J. Biol. Chem.* **240**, 1287–1293.
12. Eigner, J. & Doty, P. (1965) *J. Mol. Biol.* **12**, 549–580.
13. Paoletti, J. (1972) Thèse Doctorat es Sciences, University of Paris.
14. Yguerabide, J. (1972) in *Methods in Enzymology*, eds. Hirs, C. H. W. & Timasheff, S. N. (Academic Press, New York), Vol. XXVI, pp. 498–578.
15. Revet, B., Schmir, M. & Vinograd, J. (1971) *Nature New Biol.* **229**, 10–23.
16. Le Pecq, J. B. & Paoletti, C. (1967) *J. Mol. Biol.* **27**, 87–106.
17. Michelson, A. M., Monny, C. & Kovoor, A. (1972) *Biochimie* **54**, 1129–1136.
18. Crothers, D. M. (1968) *Biopolymers* **6**, 575–584.
19. Bauer, W. & Vinograd, V. (1970) *J. Mol. Biol.* **47**, 419–435.
20. Davidson, N. (1972) *J. Mol. Biol.* **66**, 307–309.
21. Waring, M. J. & Wakelin, L. P. G. (1974) *Nature* **252**, 653–657.
22. Wang, J. C. (1974) *J. Mol. Biol.* **89**, 783–801.