DNA polyintercalation: comparison of DNA binding properties of an acridine dimer and trimer

Bernard Gaugain, Judith Markovits*, Jean-Bernard Le Pecq* and Bernard P. Roques

Département de Chimie Organique (ERA 613 du CNRS et U. 266 de l'INSERM), UER des Sciences Pharmaceutiques et Biologiques, 75006 Paris and **Laboratoire de Physico-Chemie Macromoléculaire (LA 147 du CNRS et U. 140 de l'INSERM), Institut Gustave Roussy, 94800 Villejuif, France*

Received 3 March 1984

The DNA binding characteristics of a mono-, di- and trimeric derivative of 9-aminoacridine were studied. The length of the linking carboxamidoalkyl chains was selected to allow bis- or tris-intercalation according to the excluded-site model. Measurements of DNA unwinding angle using closed circular DNA showed that the trimeric derivative behaves as a tris-intercalating agent. Nevertheless the increase of DNA binding affinity on going from dimer to trimer was found to be relatively small. This is probably related to the large structural constraint for DNA binding of the trimeric derivative. The nature of the linking chain for the design of high-affinity DNA poly-intercalating agents appears therefore critical.

DNA intercalation *Tris-intercalation* *Acridine* *Viscosimetry*

1. INTRODUCTION

It has been demonstrated that dimerization of DNA intercalating compounds can lead to molecules able to bind to DNA with a very high affinity constant [1–10]. Among these molecules, several potent antitumor agents have been discovered [11–17]. To obtain molecules able to bind to DNA with binding constants still higher than those of bis-intercalating agents, acridine trimers have been synthesized [18,19].

On the other hand, poly-intercalating compounds might recognize base sequences on DNA because intercalation occurs preferentially between the pyrimidine-(3′–5′)-purine sequence [20]. Side chains bearing groups with hydrogen binding capacity such as amide groups have been shown to modulate the sequence specificity of intercalating agents [21–24]. Therefore, in an attempt to obtain molecules which would have high DNA binding affinity and elicit preferential binding to specific sequences, acridine trimers with linking chains bearing amide groups at appropriate position were synthesized [25].

The length of the linking chain between the subunits of dimer and trimer was selected such that it was greater than the minimum distance (10.2 Å) allowing intercalation of the subunits through the excluded-site model [2]. Moreover, it was shown that the carbonyl group of some 9-carboxamidoethylaminoacridine derivatives was able to form a hydrogen bond with guanine [23,24]. This interaction induced a GC preferential binding of the corresponding acridine derivative. Therefore amide groups were introduced in appropriate positions of the linking chains in an attempt to confer GC specificity on poly-intercalating molecules.

The DNA binding properties of an acridine trimer are reported here and compared to those of the corresponding mono- and dimeric analogues.

2. MATERIALS AND METHODS

2.1. Materials

The structures of the acridine monomer, dimer and trimer used here are shown in fig.1 and were prepared as in [25]. DNAs from calf thymus, PM2 (Boehringer) and *Micrococcus luteus* (Sigma) were

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

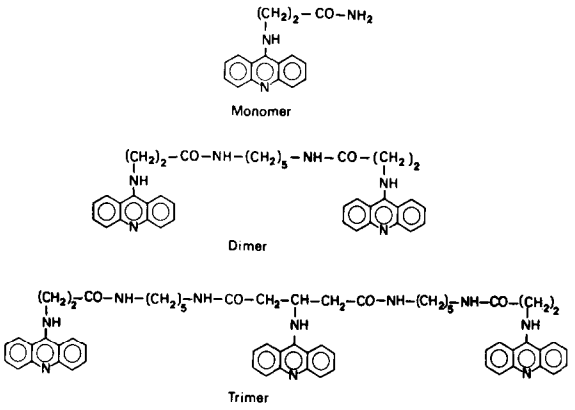

Fig.1. Chemical structure of the acridine compounds.

purified by 3 phenol extractions. Poly[d(A–T)] · poly[d(A–T)] and poly[d(G–C)] · poly[d(G–C)] (Boehringer) were used without further purification. Sonicated calf thymus DNA was prepared as in [26].

2.2. Methods

Fluorometric measurements were made in an SLM 800 spectrofluorometer with $\lambda_{exc} = 450$ nm and $\lambda_{em} = 490$ nm. The equilibrium binding constants were measured by competition experiments with ethidium dimer or ethidium bromide as in [5]. The kinetic parameters were determined with a Durrum Gibson D 110 stopped-flow instrument equipped for fluorescence detection. This apparatus was interfaced to a Minc Digital computer through a Digital MNCAA analogic Digital converter (12 bits). Data stored in the Minc computer were analyzed by non-linear regression as in [27,28].

Viscosimetric studies (measurements of length increase of short calf thymus DNA segments and unwinding angle of the PM2 DNA helix caused by the binding of the different derivatives) were performed at 25°C as in [26,29].

3. RESULTS

3.1. Viscosimetric studies

All measurements were performed at pH 5.5 because the trimer and the dimer tended to precipitate at pH > 6. The mono-, bis- or tris-intercalation ability of different derivatives can be shown by two measurements, the length increase of short DNA helices and of the unwinding angle of the DNA helix resulting from the binding of these molecules. The results are shown in table 1. It is clear that in this series of 9-aminoacridine derivatives, mono-, di- and triacridine molecules behave as mono-, bis- and tris-intercalating agents respectively. This conclusion results mainly from the variation of the unwinding angle with the number of intercalating rings in the molecule. From the length increase measurement, it is more difficult to differentiate between the effect of the dimer and trimer.

3.2. DNA binding affinity

Apparent binding constants for calf thymus DNA were determined by either competition with ethidium bromide or with ethidium dimer as in [5]. The results are shown in table 2.

The poly[d(A–T)] · poly[d(A–T)] DNA binding affinity can also be deduced from kinetic association dissociation measurements. The dissociation rate is estimated from the rate of exchange from polynucleotides to *M. luteus* or calf thymus DNAs as in [27]. The exchange kinetics followed a two-exponential process. The values of the respective amplitudes and rate constants are shown in table 2. An apparent binding constant was determined by computing the ratio of the on-rate constant over the average exchange rate computed according to [30]. The on-rate constant was equally measured

Table 1

Viscosimetric experiments

Compounds	ϕ (°)[a]	Slope[b]
Monomer	17	2.6
Dimer	32	7
Trimer	55	7.5

[a] Unwinding angle ϕ is derived from viscosimetric measurement on closed circular DNA as in [29], using 26° for the unwinding angle of ethidium [33]

[b] The lengthening of the DNA helix is proportional to the slope of the function $\log(\eta/\eta_0)$ *vs* $\log(1 + r)$ [26], where η and η_0 are, respectively, the intrinsic viscosity of sonicated DNA in the presence and absence of dye, and r is the ratio of the molar concentration of bound dye to the molar concentration of DNA base pairs. This slope is expected to be between 2 and 3, 4 and 6 and 6 and 9 for mono-, bis- and tris-intercalating agents, respectively

Table 2

DNA binding parameters of dimer and trimer

	$(K_{eq})_{app}$ (M^{-1})	k_{ex} (s^{-1})	k_1/k_{ex} (M^{-1})
Monomer	5×10^4	[a]	–
Dimer	1×10^7	1.8	1.7×10^7
Trimer	2×10^7	0.17	1.7×10^8

[a] k_{ex} was too fast to be measured in these conditions

$(K_{eq})_{app}$ is the apparent binding constant deduced from competition experiments with ethidium dimer using calf thymus DNA. k_{ex} is the rate of exchange of the dye from poly[d(A–T)] to calf thymus DNA. k_1 is the on-rate binding constant ($3 \times 10^7 M^{-1} \cdot s^{-1}$). All measurements were performed in 0.1 M Na acetate buffer (pH 5.5)

for poly[d(A–T)]·poly[d(A–T)] in the stopped-flow apparatus with fluorescence detection. The kinetics followed a single exponential. The on-rate constant was found to be in all cases in the range $2–4 \times 10^7 M^{-1} \cdot s^{-1}$. These values are very close to those found in our studies on the kinetics of interactions of mono- and diacridines [27]. The sequence specificity of these derivatives was tested by measuring the apparent binding affinity on several synthetic polynucleotides of various sequences and on DNA of different base components. No evidence of base specificity could be observed (not shown).

4. DISCUSSION

The demonstration of bis-intercalation for dimeric molecules has generally been unambiguous. DNA unwinding angles or DNA length increase differ by a factor of 2 between mono- and bis-intercalators and those changes are easily measured. Trimeric molecules can potentially intercalate one, two or three of their intercalating rings and can behave as mono-, bis- or tris-intercalating compounds. Distinction between bis- and tris-intercalating behavior might be difficult in some cases, because the variation of unwinding and DNA length increase parameters are not strictly proportional to the number of DNA intercalated rings. In the case of the molecules studied here, tris-intercalation can nevertheless be clearly established for the trimeric derivative.

Tris-intercalation was recently observed for a 9-aminoacridine derived trimer with a short (~7 Å) spacing between the aromatic rings [18]. Obviously, tris-intercalation of this compound requires intercalation of subunits between adjacent base pairs. The spacing (about 10.2 Å) between aromatic rings in the trimer studied here is longer than in the preceding case. The long-chain trimer has therefore the potential ability to bind to DNA in such a way that the intercalated rings are separated by one or two base pairs. These extended possibilities of DNA poly-intercalating mode shown by 9-aminoacridine derivatives are very likely related to the comparatively small size of the planar heterocyclic ring [23,24].

The calf thymus DNA apparent binding constant increases by a relatively small factor on going from di- to tri-meric molecules. This increase is much less than the maximum computed values since, theoretically, the maximum DNA binding constants of dimer and trimer are approximately equal to the binding constant of the monomer elevated to the second and third power, respectively [31].

The comparison of kinetic binding parameters for synthetic polynucleotides shows also that the binding affinities of dimers and trimers are not very different. These results underline the importance of the nature of linking chains, because the corresponding dimeric acridines with positively charged polyamine chains possess binding affinities several orders of magnitude higher [2,27,31].

In addition, introduction of rigid carboxamido groups into the linking chain probably induces strong structural constraint for DNA tris-intercalation decreasing therefore the trimer affinity by unfavorable entropic factors. Therefore, the structure of the linking chains might be quite critical in the design of tris-intercalating derivatives able to bind to DNA with a really high binding affinity.

The property of the linking chain also appears quite critical for obtaining dimeric molecules with antitumor properties in the series of 7H-pyridocarbazole [16]. It remains to be shown that the obtaining of trimers in these series would lead to compounds of enhanced pharmacological activity.

Such studies are now in progress in our laboratories. Finally the structural constraint on the linking chain in the DNA complex could explain the lack of sequence specificity of these molecules. In-

deed, an accurate positioning of the carboxamide group of the linking chain in the DNA groove is necessary to permit formation of the corresponding hydrogen bonds with the amino group of guanine [24].

ACKNOWLEDGEMENTS

The excellent techical assistance of J. Couprie and A. Vilar has been very much appreciated. Support of this research through grants from University Pierre et Marie Curie (Paris VI), Université René Descartes (Paris V), CNRS, INSERM, Délégation à la Recherche sur le Cancer (A.R.C., Villejuif) is gratefully acknowledged.

REFERENCES

[1] Waring, M.J. and Wakelin, L.P.G. (1974) Nature 252, 653–657.
[2] Le Pecq, J.B., Le Bret, M., Barbet, J. and Roques, B.P. (1975) Proc. Natl. Acad. Sci. USA 72, 2915–2919.
[3] Wakelin, L.P.G. and Waring, M.J. (1976) Biochem. J. 721–740.
[4] Canellakis, E.S., Shaw, Y.H., Hanners, W.C. and Schwarz, R.A. (1976) Biochim. Biophys. Acta 418, 277–289.
[5] Gaugain, B., Barbet, J., Capelle, N., Roques, B.P., Le Pecq, J.B. and Le Bret, M. (1978) Biochemistry 17, 5079–5088.
[6] Kuhlmann, K.F., Charbeneau, N.J. and Mosher, C.W. (1978) Nucleic Acids Res. 5, 2629–2641.
[7] Becker, M.M. and Dervan, B.P. (1979) J. Am. Chem. Soc. 101, 3664–3666.
[8] Lown, J.W., Gunn, B.C., Chang, R.Y., Mjumdar, K.C. and Lee, J.S. (1978) Can. J. Biochem. 56, 1006–1015.
[9] Huang, C.H., Mong, S. and Crooke, S.T. (1980) Biochemistry 19, 5537–5541.
[10] King, H.D., Wilson, W.D. and Gabbay, E.J. (1982) Biochemistry 21, 4982–4989.
[11] Canellakis, E.S., Fico, R.M., Sarris, A.H. and Shaw, Y.H. (1976b) Biochem. Pharmacol. 25, 231–236.
[12] Roques, B.P., Le Pecq, J.B., Pelaprat, D. and Le Guen, I. (1978) French Patent, 78.23.801.
[13] Roques, B.P., Pelaprat, D., Le Guen, I., Porcher, G., Gosse, C. and Le Pecq, J.B. (1979) Biochem. Pharmacol. 28, 1811–1815.
[14] Chen, T.K., Fico, R. and Canellakis, E.S. (1978) J. Med. Chem. 21, 868–874.
[15] Cain, B.F., Baguley, B.C. and Denny, W.A. (1978) J. Med. Chem. 21, 651–668.
[16] Pelaprat, D., Delbarre, A., Le Guen, I., Roques, B.P. and Le Pecq, J.B. (1980) J. Med. Chem. 23, 1336–1343.
[17] Okhuma, H., Sakai, F., Nishiyama, Y., Ohbayashi, M., Imanishi, H., Konishi, M., Miyaki, T., Koshiyama, H. and Kawaguchi, H. (1980) J. Antibiot. 33, 1087.
[18] Atwell, G.J., Leupin, W., Twigden, S.J. and Denny, W.A. (1983) J. Am. Chem. Soc. 105, 2913–2914.
[19] Hansen, J.B. and Buchardt, O. (1983) J. Chem. Soc. Chem. Commun. 162–164.
[20] Reinhardt, C.G. and Krugh, T.R. (1978) Biochemistry 17, 4845–4854.
[21] Seeman, N.C., Rosenberg, J.M. and Rich, A. (1976) Proc. Natl. Acad. Sci. USA 73, 804–808.
[22] Hélène, C. (1977) FEBS Lett. 74, 10–13.
[23] Gaugain, B., Markovits, J., Le Pecq, J.B. and Roques, B.P. (1981) Biochemistry 20, 3035–3042.
[24] Markovits, J., Gaugain, B., Barbet, J., Roques, B.P. and Le Pecq, J.B. (1981) Biochemistry 20, 3042–3048.
[25] Gaugain, B. (1981) Doctorat d'Etat és Sciences, Univ. P.M. Curie, Paris VI.
[26] Saucier, J.M., Festy, B. and Le Pecq, J.B. (1971) Biochimie 53, 973–980.
[27] Capelle, N., Barbet, J., Dessen, Ph., Blanquet, S., Roques, B.P. and Le Pecq, J.B. (1979) Biochemistry 15, 3354–3362.
[28] Markovits, J., Ramstein, J., Roques, B.P. and Le Pecq, J.B. (1983) Biochemistry 22, 3231–3237.
[29] Revet, B., Schmir, M. and Vinograd, J. (1971) Nat. New Biol. 229, 10–13.
[30] Schwartz, H.S. (1968) Rev. Mod. Phys. 40, 206–218.
[31] Markovits, J., Gaugain, B., Roques, B.P. and Le Pecq, J.B. (1981) Intermol. Forces 285–298.
[32] Markovits, J. (1980) Doctorat d'Etat ès Sciences, Univ. P.P. Curie, Paris VI.
[33] Wang, J.C. (1974) J. Mol. Biol. 89, 783–801.