JOURNAL OF PHYSICAL ORGANIC CHEMISTRY, VOL. 5, 461–466 (1992)

SURVEY OF THE DNA BINDING PROPERTIES OF NATURAL AND SYNTHETIC POLYAMINO COMPOUNDS

KENT D. STEWART* AND THOMAS A. GRAY

Department of Chemistry and Winship Cancer Center, Emory University, Atlanta, Georgia 30322, USA

Using a fluorescence-detected ethidium displacement assay, the calf thymus DNA complexation properties of 27 mono-, di-, tri-, tetra- and hexacationic polyamines were determined. The DNA-binding affinity of these polyamine compounds increased with increasing cationic charge on the polyamine. Although most of the compounds exhibited no base pair binding selectivity, two of the tricationic polyamines possessing additional neutral amine groups exhibited approximately tenfold GC binding selectivities.

INTRODUCTION

One of the goals of DNA complexation studies is to develop a sufficient understanding of the binding forces to allow the design of novel DNA binding agents.[1] The conformational flexibility of the receptor and the existence of multiple binding modes and sites complicate the design process. One particularly challenging goal is the design of molecules which bind to DNA in the major groove. Whereas examples of biopolymers (nucleic acids[2] or proteins[3]) which bind in the major groove may be cited, no unambiguous examples of 'organic' compounds which bind in the major groove exist. For example, the polyamine class of compounds (spermine, spermidine, etc.) binds to DNA in a non-intercalative manner, however, the exact position (or positions) of the binding interaction (major groove, minor groove, or phosphate backbone) has not been established.[4] Three crystal structures of polyamines bound to DNA oligomers indicate that well defined groove-binding orientations can be achieved in the solid state.[5–7] In the case of the B-DNA conformation, spermine was observed to span the major groove.[5] Accordingly, we embarked on a general study of polyamine–DNA interactions to establish rules that might be useful in future design of DNA complexation agents for major groove binding. In this paper, we report on our results with 27 polyamines.

EXPERIMENTAL

Polyamines **1–24** (Table 1) were purchased from Aldrich Chemical and used as received. Compounds **24–27** were prepared from the corresponding aryldibromides or aryltribromides by procedures analogous to published procedures.[8] All new compounds gave satisfactory nuclear magnetic resonance, mass spectral and combustion analysis data.

DNA-binding studies. Calf thymus DNA (CT-DNA) was a generous gift from Professor W. D. Wilson and was purified according to a literature procedure.[9] Poly $d(AT)_2$ and poly $d(GC)_2$ were purchased from Sigma Chemical. The experimental details of the ethidium displacement assay have been discussed in the literature.[10,11] The C_{50} value refers to the concentration of test compound required to displace ethidium from DNA under a given set of assay conditions (2 mM HEPES, 8 mM NaCl, 0·05 mM EDTA pH 7·0, [ethidium] = 1·26 μM, [DNA] = 1 μM, 25 ± 0·3 °C) as measured by fluorescence spectroscopy. DNA thermal denaturation studies and viscometric studies were carried out using literature procedures.[12] All measurements were made in duplicate. Error limits on C_{50} values are ± 10% and on ΔT_m values ± 1 °C.

RESULTS AND DISCUSSION

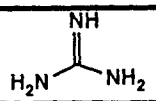
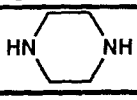
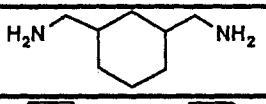
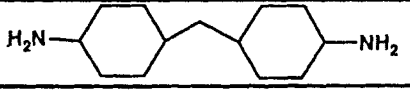
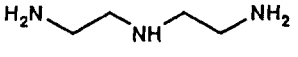
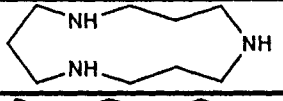
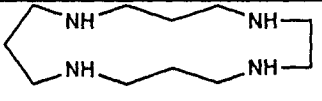
The direct study of polyamine–DNA binding interactions is made difficult by the lack of an ultraviolet or visible light chromophore in the polyamine compounds. In this work, polyamine binding was measured indirectly by monitoring their displacement of the intercalator ethidium from DNA. The ethidium displacement assay has been used to assess the DNA binding

* Author for correspondence. Present address: Division of Organic Chemistry, Burroughs Wellcome Company, 3030 Cornwallis Drive, Research Triangle Park, NC 27709, USA.

0894–3230/92/080461–06$08.00

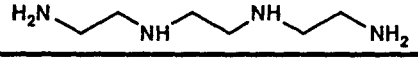
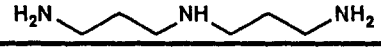
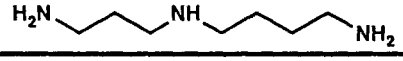
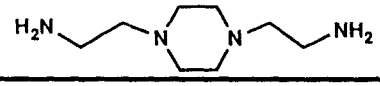
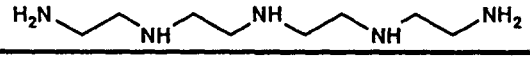
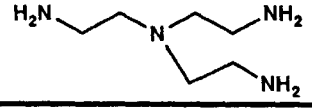
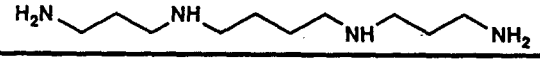
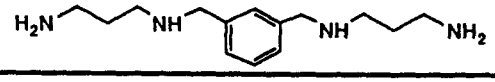
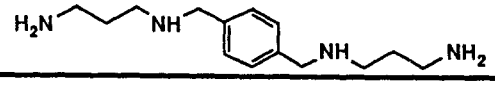
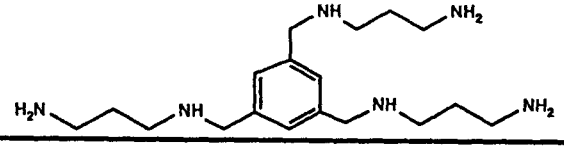
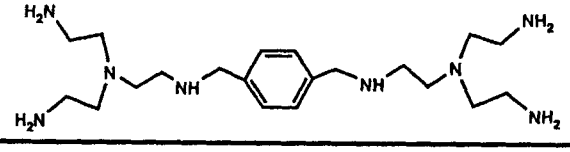
Received 15 January 1992
Revised 20 April 1992

Table 1. Electrostatic charge and C_{50} values for polyamine binding to calf thymus DNA

	COMPOUND	Charge in Solution pH = 7.0	C_{50} Value CT-DNA (μM)
1		+1	28,000
2		+1	29,000
3	$H_2N—(CH_2)_2—NH_2$	+2	2,500
4	$H_2N—(CH_2)_4—NH_2$	+2	2,300
5	$H_2N—(CH_2)_6—NH_2$	+2	2,700
6	$H_2N—(CH_2)_8—NH_2$	+2	1,400
7	$H_2N—(CH_2)_{10}—NH_2$	+2	1,100
8	$H_2N—(CH_2)_{12}—NH_2$	+2	300
9	$(CH_3)_3N^+—(CH_2)_{10}—N(CH_3)_3^+$	+2	12,000
10		+2	1,800
11		+2	2,100
12		+2	2,000
13		+2	2,400
14		+2/+3	230

continued

Table 1. (*Continued*)

15 	+2/+3	140
16 	+3	36
17 	+3	41
18 	+3	180
19 	+3	20
20 	+3	17
21 	+3	22
22 	+3	31
23 	+4	1.6
24 	+4	2.2
25 	+4	1.9
26 	+6	0.15
27 	+6	0.13

strengths of a number of classes of compounds, including both intercalators and groove binders.[11] The C_{50} binding value determined in this measurement has been shown to correlate with the dissociation constant for the polyamine class of compounds.[8,13,14] Interpretation of the C_{50} values in terms of a well defined binding process is not possible, however. Displacement of ethidium from DNA could result from polyamine binding in the major or minor groove or along the phosphate backbone. Also, conformational changes in the DNA induced by the polyamine would not be apparent in this assay. With these caveats, the ethidium displacement assay is still appropriate for screening groups of compounds to discover binding trends. Our work was focused on making relative comparisons of C_{50} values. The structures and C_{50} values (in μM units) of polyamines **1**–**27** binding to calf thymus DNA are shown in Table 1.

The monocationic amines guanidine and piperazine (**1** and **2**) exhibit the weakest binding of all of the amines studied (a high C_{50} value indicates a low DNA affinity). The C_{50} values listed in Table 1 are only rough estimates of the DNA binding affinity of these polyamines because the binding titration concentrations (10–100 mM) are well above the buffer salt concentration (10 mM).

A chain length dependence was observed in the binding of α,ω-diamine compounds **3**–**8** binding to DNA. While the C_{50} values for ethanediamine (**3**) putrescine (**4**) and hexanediamine (**5**) binding to CT-DNA are similar, there is a slight increase in binding strength for octanediamine (**6**) and decanediamine (**7**) and a significant increase for dodecanediamine (**8**). This chain length dependence has been reported previously in thermal denaturation studies of DNA.[15] Interestingly, the distance between opposing phosphates in duplex B-DNA (19 Å) is similar to the distance between charged amines in the fully extended dodecanediamine. A binding interaction of dodecanediamine spanning the major groove analogous to that observed in the crystal structure[5] of spermine bound to B-DNA may be envisioned, although this proposed complex requires further experimental support. Incorporation of cyclohexane rings in the aliphatic backbone of the diamino compounds **10** and **11** did not alter the binding affinity significantly.

The DNA-binding affinity of the bis-quaternary amine **9** is much weaker than the corresponding decanediamine **7**. Even though these two compounds possess equal chain length, a tenfold difference in C_{50} values was observed. While hydrogen bonding interactions with DNA are not possible for compound **9**, electrostatic interactions may also be diminished.

The reported pK_a values for diethylenetriamine (**12**) are 9·9, 9·1 and 4·3, and therefore at pH7 the compound is dicationic.[16] As shown in Table 1, the C_{50} value for compound **12** binding to CT-DNA is similar to the values determined for compounds **4** and **5**, indicating that the uncharged amine group in **12** has little effect on the CT-DNA binding affinity.

Incorporation of the polyamino system into a macrocylic ring, as in compounds **13** and **14**, did not significantly alter the binding affinity from that seen for other di- or tricationic amines. A more precise interpretation of the results for **13** and **14** is made difficult because results for suitable open-chain compounds are not available.

The reported pK_a values for triethylenetetraamine (**15**) are 10·0, 9·3, 6·9 and 3·7, and therefore at pH 7 the compound exists in a number of protonated states with the net charge close to +2·5.[17] In agreement with this analysis, the C_{50} value for compound **15** binding to CT-DNA is between that for putrescine (**4**), +2, and spermidine (**17**), +3.

The tricationic polyamines **16**–**22**, including spermidine (**17**), exhibit a greater affinity for DNA than the dicationic amines. Although not listed in Table 1, most of the polyamines studied in this work exhibited similar C_{50} values (within a factor of 2) when calf thymus DNA was replaced with poly d(AT)$_2$ or *poly* d(GC)$_2$. These polyamines show very little base-pair binding selectivity. Two notable exceptions are compounds **19** and **20**. The C_{50} values for **19** and **20** binding to poly d(AT)$_2$ were 55 and 50 μM, respectively, while the values for binding to poly d(GC)$_2$ were 5·3 and 6·0 μM, respectively. The lower numbers with poly d(GC)$_2$ indicated that these two compounds preferentially bind to GC rather than AT base pairs with ten- and eightfold selectivity, respectively. The reported pK_a values for **19** are 10·7, 10·0, 8·5 and 5·8 and those for **20** are 10·0, 9·2, 8·2, 4·1 and 2·6.[17,18] In neutral aqueous solutions, compounds **19** and **20** would be expected to be triprotonated and to possess neutral amine groups. Perhaps the presence of neutral amine groups in these two compounds allows other binding interactions to occur in addition to the electrostatic interactions. While electrostatic interactions are certainly dominant for the binding of **19** and **20**, an interaction such as hydrogen bonding with the guanine N-7 or cytosine 4-NH$_2$ group might cause the GC binding selectivity.[8] Further investigation of these observations is in progress.

The tetracationic polyamines **23**–**25**, including spermine (**23**), exhibit C_{50} values approximately ten times lower than the tricationic polyamines, indicating that the binding to DNA is stronger than the tricationic polyamines. The novel hexamine compounds **26** and **27** are each assumed to be hexacationic at neutral pH based on comparisons of their structures with other amine compounds.[19] These two compounds exhibit C_{50} values ten times lower than the tetracationic compounds, indicative of high DNA affinity.

To confirm the tight DNA binding of the hexaamino compound **27** observed in the ethidium displacement assay, thermal denaturation studies were performed

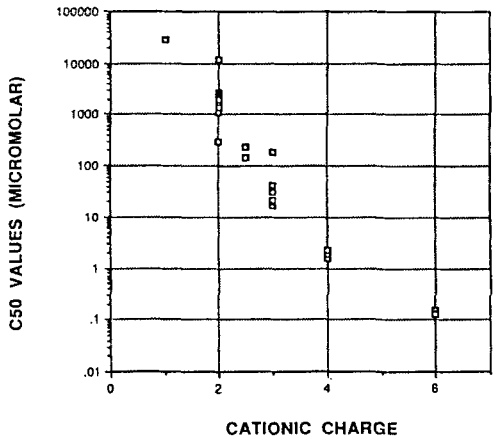


Figure 1. Plot of C_{50} versus electrostatic charge for the 27 polyamines listed in Table 1

with putrescine, spermidine, spermine and compound **27** and values of ΔT_m of 2, 5, 12 and 16 °C, respectively, were determined. Hence there was a correlation between C_{50} values and ΔT_m values for these four polyamines. In addition, a viscometric titration of **27** indicated that the compound with its single aromatic ring does not intercalate into DNA.

The C_{50} values in Table 1 were plotted against the charge for all the polyamines studied. Charges at neutral pH were assigned by comparison with compounds with known pK_a values.[19] Two of the compounds, **14** and **15**, were assigned charges of +2·5 because they had estimated pK_a values close to 7. The resultant graph is shown in Figure 1. There was a linear correlation between the logarithm of the C_{50} values and the electrostatic charge of the compounds. To the extent that the C_{50} values reflect dissociation constants, the log C_{50}, and therefore the free energy of complexation, ΔG, correlated directly with charge. The same kind of correlation has been observed with oligolysine derivatives[20] and has a theoretical basis in Manning's theory of counterion condensation on polyelectrolytes.[21,22]

CONCLUSIONS

Evidence presented here and elsewhere[4,23] suggests that some compounds of polyamine structure can make site-specific interactions in the grooves of DNA. The chain-length dependence of the dicationic linear diamines and the GC base-pair selectivity of compounds **19** and **20** attest to this conclusion. Binding can be significantly enhanced by adding positive charges, as shown by the hexaamine compounds **26** and **27**. Combination of this powerful electrostatic interaction with appropriate hydrophobic and hydrogen bonding interactions should be a fruitful, albeit challenging, research strategy in the future for designing DNA groove binding agents.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Donors of the Petroleum Research Fund, administered by the American Chemical Society, the Olin Corporation Charitable Trust Grant of Research Corporation, the Emory University Research Program and the Winship Cancer Clinic of Emory University. The NMR and mass spectrometers used in these studies were made possible through NSF and NIH equipment grants. The authors thank M. Cory and T. Fairley for carrying out the thermal denaturation and viscometric experiments.

REFERENCES

1. P. E. Nielsen, *Bioconjugate Chem.* **2**, 1–12 (1991).
2. H. E. Moser and P. B. Dervan, *Science (Washington, D.C.)* **238**, 645–650 (1987).
3. D. L. Ollis and S. W. White, *Chem. Rev.* **87**, 981–995 (1987).
4. B. G. Feuerstein, N. Pattibiraman and L. J. Marton, *Proc. Natl. Acad. Sci. USA*, **83**, 5948–5952 (1986).
5. H. R. Drew and R. E. Dickerson, *J. Mol. Biol.* **151**, 535–556 (1981).
6. S. Jain, G. Zon and M. Sundaralingam, *Biochemistry* **28**, 2360–2364 (1989).
7. R. V. Gessner, C. A. Frederick, G. J. Quigley, A. Rich and A. W. J. Wang, *J. Biol. Chem.* **264**, 7921–7935 (1989).
8. K. D. Stewart, *Biochem. Biophys. Res. Commun.* **152**, 1441–1446 (1988); see also K. D. Stewart, in *Proceedings of the Fifth International Symposium on Inclusion Phenomena and Molecular Recognition*, edited by J. L. Atwood, pp. 75–80. Plenum Press, New York (1989).
9. J. W. Lown, A. R. Morgan, S.-F. Yen, Y.-H. Wang and W. D. Wilson, *Biochemistry* **24**, 4028–4035 (1985).
10. B. F. Cain, B. C. Baguley and W. A. Denny, *J. Med. Chem.* **21**, 658–668 (1978).
11. B. C. Baguley, *Mol. Cell. Biochem.* **43**, 167–181 (1982).
12. M. Cory, D. D. McKee, J. Kagan, D. W. Henry and J. A. Miller, *J. Am. Chem. Soc.* **107**, 2528–2536 (1985).
13. H. S. Basu, H. C. A. Schietert, B. G. Feuerstein and L. J. Marton, *Biochem. J.* **269**, 329–334 (1990).
14. M. L. Edwards, R. D. Snyder and D. M. Stemerick, *J. Med. Chem.* **34**, 2414–2420 (1991).
15. M. Gourevitch, P. Puigdomenech, A. Cave, G. Etienne, J. Mery and J. Parello, *Biochimie* 56, 967–985 (1974).
16. J. W. Allison and R. J. Angelici, *Inorg. Chem.* **10**, 2233–2238 (1971).
17. P. Paoletti, L. Fabbrizzi and R. Barbucci, *Inorg. Chem.* **12**, 1861–1864 (1973).
18. C. N. Reilly and A. Vavoulis, *Anal. Chem.* **31**, 243–248 (1959).
19. D. D. Perrin, B. Dempsey and E. P. Serjeant, *pK$_a$ Prediction for Organic Acids and Bases.* Chapman and Hall, London (1981).

20. S. A. Latt and H. A. Sober, *Biochemistry* **6**, 3293–3306 (1967).
21. M. T. Record, T. M. Lohman and P. de Haseth, *J. Mol. Biol.* **107**, 145–158 (1976).
22. G. S. Manning, *Q. Rev. Biophys.* **11**, 179–246 (1978).
23. P. M. Vertino, R. J. Bergeron, P. F. Cavanaugh, Jr. and C. W. Porter, *Biopolymers* **26**, 691–703 (1987).