Effect of the Linking Position of a Side Chain in Bis(quinolylmethyl)ethylenediamine as a DNA Binding Agent

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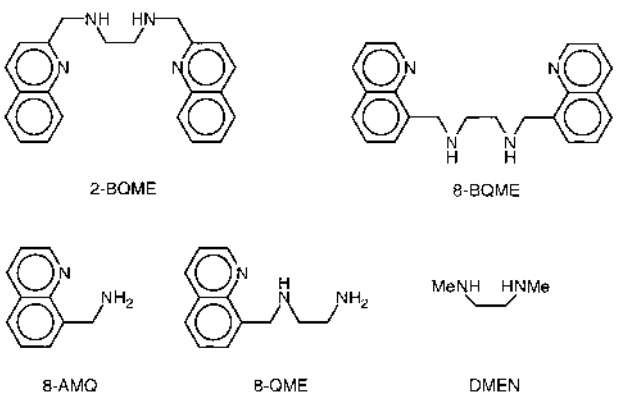
Two bisquinoline derivatives, *N*,*N*′-bis(2-quinolylmethyl)ethylenediamine (2-BQME) and *N*,*N*′-bis(8-quinolylmethyl)ethylenediamine (8-BQME) have been synthesized, and their ability to bind to duplex DNA was studied. 8-BQME bound to DNA more strongly than 2-BQME, judging from the extent of increase in the melting temperature of duplex DNA, the UV-vis spectral change, and ethidium displacement assay. These compounds exhibited apparent AT-specificity suggesting minor groove binding in addition to intercalation.

Key words bisquinoline; ethylenediamine; DNA

Since potent DNA-binding agents are among the most promising candidates for chemotherapeutic drugs, a lot of natural and unnatural ligands for DNA have been extensively studied. Intercalators and groove binders have gathered the greatest attention in this area. When compared with monointercalators, bisintercalators have several advantages including : i) increased binding affinity, ii) enhanced specificity for DNA due to bisintercalation, and iii) improved sequence selectivity.[1,2] Among a large number of the DNA bisintercalators studied to date, bisquinolines have potential as antibiotics, antimalarial,[3—5] and antitumor agents. In addition, these compounds are useful for studying the role of chromophores in natural compounds.[6] The quinoline ring is considered to have a low potential for both intercalating and groove binding ability.[7] However, due to its small ring size, it is expected to be capable of penetrating into cell membranes and dispersing into solid tumor tissues.[8]

As a linker of bisquinoline derivatives, an ethylenediamine moiety has several advantageous features such as donation to the phosphate backbone of DNA in a protonated structure, enhancement of water solubility, ability for DNA hydrolysis, and metal chelation ability that introduces DNA modification or scission functionality.[9] Thus, a systematic study aimed at the DNA binding ability of bisquinolines with an ethylenediamine linker seems to be important; however, such a study has not been reported to date to the best of our knowledge. In the present study, we report the synthesis and evaluation of two very simple bisquinoline derivatives, *N*,*N*′-bis(2-quinolylmethyl)ethylenediamine (2-BQME) and *N*,*N*′-bis(8-quinolylmethyl)ethylenediamine (8-BQME) as DNA binding agents by DNA-melting, spectroscopic titration, and ethidium displacement assay.



2- and 8-BQME were synthesized from the corresponding methylquinoline *via* bromination of the methyl groups, followed by dimerization using ethylenediamine. As reference compounds, 8-(aminomethyl)quinoline (8-AMQ), *N*-(8-quinolylmethyl)ethylenediamine (8-QME) and *N*,*N*′-dimethylethylenediamine (DMEN) were used in this study. All compounds were isolated as hydrochloride salts.

Figures 1a and 1b show the UV-melting curves of calf thymus DNA, demonstrating the enhanced thermal stability of the duplex in the presence of 2- and 8-BQME, respectively. Increasing the concentrations of both ligands leads to stabilization of the duplex. The thermal denaturation temperatures (T_m) are summarized in Table 1.

Although 8-AMQ did not show any obvious duplex stabilization, 8-BQME exhibited a significant effect. Comparing the 8-AMQ and 8-QME, the extra aliphatic nitrogen atom increased DNA affinity. The addition of an extra 8-quinolyl ring into DMEN and 8-QME also had an important effect. One quinoline ring corresponds to a 2 degree increase in T_m where the [drug]/[base pair of DNA] ratio (r) is 0.5. It should be noted that the addition of a 2-quinolyl group to DMEN has absolutely no effect on its binding affinity to DNA, since the ΔT_m values of 2-BQME and DMEN are almost identical in all cases. It is concluded that the 2-quinolyl substituent has a markedly smaller effect on DNA binding than the 8-quinolyl moiety.

These quinoline compounds exhibited their absorption maxima at 313—315 nm, and with the addition to calf thymus DNA, these absorption bands slightly decreased (Fig. 2). However, distinct isosbestic points were not observed and the spectral change in the long-wavelength region arising from the quinoline ring transition was too small to determine the association constants of these quinoline derivatives. The extent of spectral change in quinoline derivatives was parallel with the DNA-stabilizing ability obtained from UV-melting experiments (8-BQME>8-QME>2-BQME).

From the result of a duplex denaturation experiment and

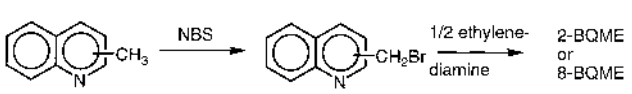


Chart 1

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DNA-titration analysis, the quinoline ring moiety should interact with DNA. These quinolines were also found to drive out DNA-bound ethidium bromide. Although ethidium bromide is not fluorescent in free solution, it becomes fluorescent when intercalated into DNA. The C_{50} values (the micromolar drug concentrations necessary to reduce the fluorescence of initially DNA-bound ethidium by 50%) against calf thymus DNA, $(poly[AT])_2$, and $(poly[GC])_2$ obtained by this assay was listed in Table 2. The extent of fluorescence quenching due to a non-displacement mechanism were estimated to be small in the experiment where excess DNA was employed. Apparent AT-specificity in both compounds was observed. A higher GC/AT ratio of the C_{50} value in 8-BQME comes from the weak interaction with $(poly[GC])_2$, suggesting a minor-groove interaction of this compound with the DNA duplex. In contrast, 2-BQME displaces ethidium in a similar manner, regardless of whether ethidium binds to calf thymus DNA or $(poly[GC])_2$. This fact suggests that electrostatic interaction governs the interaction of 2-BQME with DNA.

In conclusion, two bisquinolines with an ethylenediamine linker have been synthesized, and an 8-linked derivative was found to bind to DNA more strongly than the 2-linked derivative. AT-specific interaction of these compounds with DNA suggests the interaction mode of these compounds is primarily groove binding.

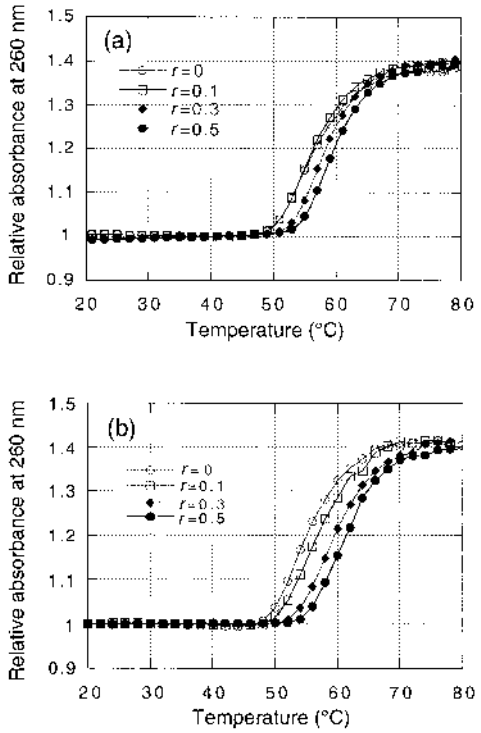

Fig. 1. UV Melting Profiles at 260 nm of Calf Thymus DNA Duplex in the Presence of Bisquinoline Derivatives

a) 2-BQME, b) 8-BQME. r=[drug]/[DNA (in base pair)].

Experimental

Melting points were determined on a micro hot-stage Yanaco MP-S3. ^{1}H-NMR were measured on a Varian Gemini 2000 spectrometer at 300 MHz. The mass spectrum was measured on a JEOL SX102 mass spectrometer. Measurement of absorbance spectra and the DNA denaturation experiment were run on a Shimadzu UV-3100PC with temperature controller SPR-8. Fluorescence measurements were performed on a Jasco FP-750 with an ETC-272T temperature controller.

8-(Bromomethyl)quinoline[10] 8-Methylquinoline (9.9 g, 69 mmol) was dissolved in CCl_4 (70 ml) and refluxed in the presence of *N*-bromosuccinimide (NBS) (12.4 g, 69 mmol) and 2,2′-azobisisobutyronitrile (AIBN)

Table 1. Increase in Melting Temperature (ΔT_m) of Calf Thymus DNA in the Presence of Quinoline Derivatives[a)]

Compounds	ΔT_m/°C		
	r=0.1	r=0.3	r=0.5
2-BQME	*ca.* 0	1.4	3.2
8-BQME	2.9	5.1	7.1
8-QME	1.4	4.2	5.7
8-AMQ	*ca.* 0	*ca.* 0	*ca.* 0
DMEN	*ca.* 0	1.5	3.1

a) Measured at 25 °C.

Table 2. C_{50} Values[a)] in the Ethidium Displacement Assay Bound to Calf Thymus DNA, $Poly[d(AT)]_2$, and $Poly[d(GC)]_2$[b)]

Compound	C_{50}/μM			C_{50}(GC)/C_{50}(AT)
	Calf Thymus	$Poly[d(AT)]_2$	$Poly[d(GC)]_2$	
2-BQME	140	105	145	1.4
8-BQME	38	35	57	1.6

a) See text. *b*) [DNA]=0.5 μM, [ethidium]=1.26 μM.

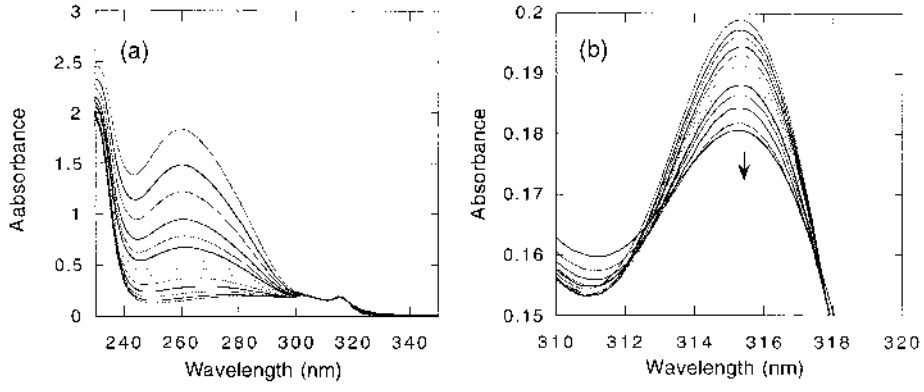

Fig. 2. Absorbance Change of 2-BQME (2.5×10^{-5} M) in the Presence of an Increasing Amount of Calf Thymus DNA in 1.0 mM Sodium Cacodylate Buffer Containing 4.0 mM NaCl (pH 6.0) at 25 °C

a) Whole spectra, b) magnified spectra of 310—320 nm region.

(0.14 g) for 2 h. After cooling, the solution was filtered, evaporated, and the residue was washed with MeOH to give 8-(bromomethyl)quinoline as white needles (11.4 g, 75%). 2-(Bromomethyl)quinoline[11,12] was obtained in a similar manner (54%).

8-BQME A mixture of ethylenediamine (0.28 g, 4.6 mmol), potassium carbonate (2.3 g, 16 mmol), and anhydrous acetone (20 ml) was stirred for several minutes at room temperature. To the mixture was added dropwise 8-(bromomethyl)quinoline (3.0 g, 14 mmol) in 30 ml of anhydrous acetone. After being refluxed overnight, the reaction mixture was dried under reduced pressure and the residue was extracted with chloroform. The solvent was dried, evaporated, and the resulting residue was dissolved in 2 N HCl (100 ml) and washed with dichloromethane (50 ml×4 times) to remove unreacted 8-(bromomethyl)quinoline. The acidic solution was adjusted with NaOH to pH=14, extracted with dichloromethane, dried, and evaporated to give 8-BQME as a yellow oil. This oil was dissolved in conc.HCl and methanol to give tetrahydrochloride salt (0.052 g, 38%), mp 180—183 °C. ^{1}H-NMR (D_2O) δ: 3.88 (s, 4H), 4.94 (s, 4H), 7.34—7.40 (m, 2H), 7.46—7.52 (m, 2H), 7.60 (d, 2H), 7.72 (d, 2H), 8.25—8.30 (m, 2H), 8.55 (d, 2H). *Anal*. Calcd for $C_{22}H_{32}Cl_4N_4O_3$ (BQME·4HCl·3H_2O): C, 48.72; H, 5.95; N, 10.33. Found: C, 48.84; H, 5.72; N, 9.99.

2-BQME was obtained in a manner similar to that used for 8-BQME (8%), mp 165—168 °C. ^{1}H-NMR (D_2O) δ: 3.58 (s, 4H), 4.50 (s, 4H), 7.40 (d, 2H), 7.5—7.62 (m, 4H), 7.7—7.9 (m, 4H), 8.28 (d, 2H). HR-MS (FAB) Calcd for $C_{22}H_{23}N_4$ (M+H) 343.1923. Found 343.1892.

DNA Denaturation Experiment Absorbance *vs*. temperature melting curves were measured at 260 nm using 5×10^{-5} M (in base pairs) of calf thymus DNA in 1.0 mM sodium cacodylate buffer containing 4.0 mM NaCl (pH 6.0). The heating rate was 3 °C/min. T_m values were determined from the first derivative plots of melting curves.

Spectroscopic Analysis An absorbance change in bisquinolines ([compound]=2.5×10^{-5} M) was monitored in the presence of duplex DNA (2—25 $\times10^{-5}$ M (in base pairs)) in 1.0 mM sodium cacodylate buffer (pH 6.0) at 25 °C.

Ethidium Displacement Assay C_{50} values (the micromolar drug concentrations necessary to reduce the fluorescence of initially DNA-bound ethidium by 50%) were obtained using 0.5 μM (in base pairs) of DNA (1.0 μM sodium cacodylate buffer containing 4.0 μM NaCl (pH 6.0)) with 1.26 μM of ethidium at 25 °C. The extent of fluorescence quenching due to a non-displacement mechanism was estimated by the similar methods employing 20 μM of DNA and 2 μM of ethidium.

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References

1) Kim H. K., Kim J.-M., Kim S. K., Rodger A., Nordén B., *Biochemistry*, **35**, 1187—1194 (1996).
2) Molina A., Vaquiero J. J., Garcia-Navio J. L., Alvarez-Builla J., de Pascual-Teresa B., Gago F., Rodorigo M. M., Ballesteros M., *J. Org. Chem*., **61**, 5587—5599 (1996).
3) Nasr M., Burckhalter J. H., *J. Heterocyclic Chem*., **16**, 497—500 (1979).
4) Vannerstrom J. L., Ellis W. Y., Ager, A. L., Jr., Andersen S. L., Gerena L., Milhous W. K., *J. Med. Chem*., **35**, 2129—2134 (1992).
5) Raynes K., Galatis D., Cowman A. F., Tilley L., Deady L. W., *J. Med. Chem*., **38**, 204—206 (1995).
6) Alfredson T. V., Maki A. H., Warning A. J., *Biochemistry*, **30**, 9665—9675 (1991).
7) Mikata Y., Yokoyama M., Ogura S., Okura I., Kawasaki M., Maeda M., Yano S., *Bioorg. Med. Chem. Lett*., **8**, 1243—1248 (1998).
8) Denny W. A., Rewcastle G. W., Bauguley B. C., *J. Med. Chem*., **33**, 814—819 (1990).
9) Dervan P. B., Becker M. M., *J. Am. Chem. Soc*., **100**, 1968—1970 (1978).
10) Bu X. H., An D. L., Chen Y. T., Shionoya M., Kimura E., *J. Chem. Soc., Dalton Trans*., **1995**, 2289—2295.
11) Rukachaisirikul V., Koert U., Hoffmann R. W., *Tetrahedron*, **48**, 4533—4544 (1992).
12) Brown B. R., Hammick D. L., Thewlis B. H., *J. Chem. Soc*., **1951**, 1145—1148.