Asymmetric intercalation of N^1-(acridin-9-ylcarbonyl)spermine at homopurine sites of duplex DNA

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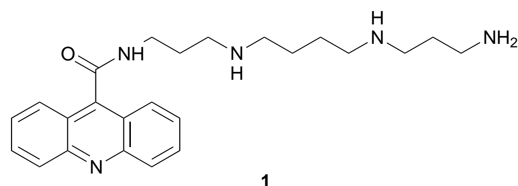
N^1-(Acridin-9-ylcarbonyl)spermine binds at 5′-pu-p-pu..5′-py-p-py sites of DNA with the acridine moiety asymmetrically intercalated, stacked between the two purine bases; the spermine moiety interacts with the homopyrimidine phosphodiester backbone of the intercalation site and protects against DNase I cleavage of this backbone, but does not protect against cleavage of the homopurine backbone at the same intercalation site.

Acridine, a common DNA intercalator, has been used extensively to modulate the DNA binding properties of many ligands through covalent attachment of the acridine moiety. Examples include acridine conjugates with peptides,[1] oligodeoxynucleotides,[2] minor-groove DNA binders such as netropsin and distamycin[3] and the DNA alkylating agent cisplatin.[4,5] The binding selectivity of these conjugates generally reflects that of the non-acridine part of the molecule, although acridine conjugation seems to redirect the alkylation selectivity of some aniline mustards.[6] The site-specific photocleavage of DNA by acridine- 9-carboxamide–imidazole conjugates, in association with Co^{III}, may be a result of an acridine preference for intercalation at a 5′-GpG..5′-CpC site.[7]

Polyamines, such as spermine and spermidine, are polycationic molecules that have limited DNA-sequence selectivity, but may preferentially bind to GC-rich major grooves,[8] and AT-rich minor grooves.[9] Amongst many examples of polyamine conjugates[10] are those having polyamines covalently bound to long-chain lipids,[11] anthracene[12] and mustard alkylating agents such as chlorambucil.[13] There is no apparent evidence of the polyamine introducing a sequence-selective binding effect into any of these molecules, but in each it is believed that the polyamine enhances the binding affinity through electrostatic interactions with DNA.

To combine the intercalating effects of acridine and the high affinity polyamine binding to DNA, we have synthesized N^1-(acridin-9-ylcarbonyl)spermine **1**.‡ To investigate any possible sequence selectivity of **1**, we then used a DNase I protection assay with the Hind III/Nhe I restriction fragment of pBR322 plasmid duplex DNA.§ The protection from DNase I cleavage by **1** was pronounced at eight sites within the readable window of the gel (approximately from base-pair 81 to 150, Fig. 1). Of these, seven are homopyrimidine tracts of two or more bases. The remaining site corresponds to a 5′-CpG..5′-CpG sequence (site 5) and was only weakly protected. Of the pyrimidine tract sites, five span only two pyrimidine bases, with both 5′-CpC (sites 1 and 3, with weak protection at site 4) and 5′-TpT (sites 6 and 7) equally protected. Sites 2 and 8 contain longer 5′-CpCpT and 5′-CpTpTpT tracts, respectively, both of which are fully protected. Hence, the nature of the pyrimidine (C or T) seems to be unimportant in establishing protection against DNase I by polyamine amide **1**. In addition to the pyrimidine protection sites, some enhancement of cleavage is observed adjacent to several protection sites (for example, to the 3′ side of site 7). Such enhancement has been observed previously in this assay,[3] and could be the result of a DNA conformational change brought about by the binding of **1**.



In the DNase I assay we found no protection of purine tracts on the bottom strand (italicised in Fig. 1). This is an interesting result, given that such tracts have corresponding complementary pyrimidine tracts in the top strand. One might anticipate that the ligand binding causing the protection described above would also occur in these regions of the DNA, and would offer similar protection to the homopurine regions of the bottom strand. We conclude from this that there is an asymmetric binding of polyamine amide **1** to the homopurine..homopyrimidine DNA, such that only the pyrimidine backbone is protected from DNase I.

To investigate further this unusual experimental result, computer simulations were performed using 18-mer duplexes containing intercalation sites[12] corresponding to two protection sites. These were site 3 (intercalation site between base pairs

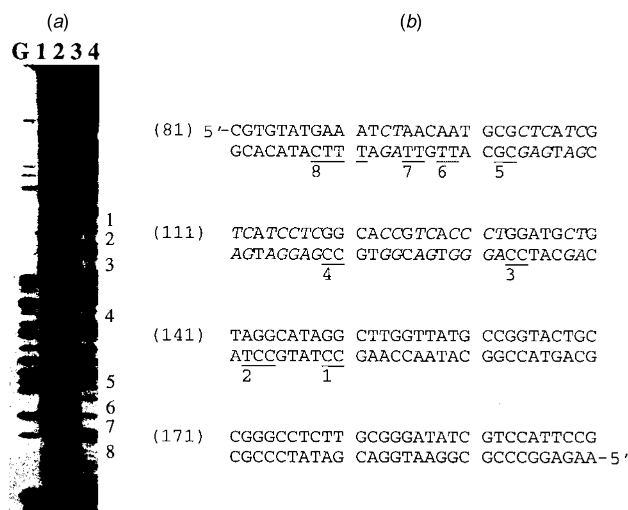


Fig. 1 Sites of DNase I protection by N^1-(acridin-9-ylcarbonyl) spermine **1** in a 3′-end [^{32}P]-bottom strand labelled Hind III/Nhe I restriction fragment of pBR322. (*a*) Autoradiogram showing Maxam–Gilbert dimethyl sulfate–piperidine mediated guanine specific cleavage (lane G), and DNase I cleavage in the presence of no polyamine amide **1** (lane 1), 0.2 μM **1** (lane 2), 1.0 μM **1** (lane 3) and 5.0 μM **1** (lane 4). Protection sites are numbered 1 to 8 in the 5′ to 3′ direction of the bottom strand. (*b*) Sequence of restriction fragment corresponding to the autoradiogram. Numbered and underlined sequences show protection sites; note that no protection was observed at italicised sequences.

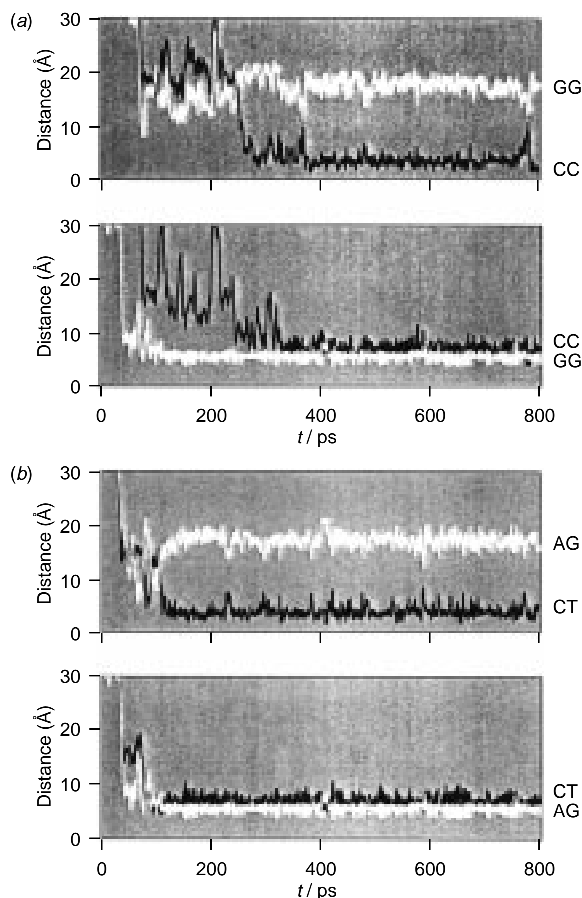


Fig. 2 Simulations of polyamine amide **1** with 18-mer DNA molecules having (a) a central 5′-GpG..5′-CpC intercalation site (site 3) and (*b*) a central 5′-ApG..5′-CpT intercalation site (site 2), showing in the upper panels of (*a*) and (*b*) the polyamine contact distances to the phosphates of the respective homopyrimidine and homopurine backbones, and in the lower panels the acridine contact distances to the C1′ atoms of the same backbones

133 and 134, 5′-GpG..5′-CpC in a sequence corresponding to base pairs 125 to 142) and site 2 (intercalation site between base pairs 142 and 143, 5′-ApG..5′-CpT, in a sequence corresponding to base pairs 134 to 151). Molecular dynamics simulations of the interactions of **1** with each site were performed in the AMBER4.1 forcefield using similar conditions to those described elsewhere.[12] Simulations were performed for 800 ps and the DNA motion was frozen throughout the calculations.[12] The ligand was initially positioned about 20 Å from the DNA, allowing it to move to the DNA, bind and then intercalate from either groove with a random orientation of the polyamine moiety.[12]

In Fig. 2 we show the results of these simulations. The data shown in the upper panels of Fig. 2(*a*) and (*b*) are the distances of the polyamine moiety to the phosphodiesters of each intercalation site, using, at each time point, the phosphate in each strand closest to the 'centre' of the polyamine (defined as the midpoint of the two secondary amines). In the lower panels, the distance from the centre of the acridine (pyridine) ring to the C1′ atoms of the deoxyriboses of each intercalation site, using, at each time point, the C1′ atom of each strand closest to the acridine centre. Following intercalation (after 300 ps for site 3 and 100 ps for site 2, from the major groove for each site), the acridine moiety remains closer to the homopurine strand for both sites. Conversely, whilst the acridine moiety is intercalated, the polyamine interacts only with the homopyrimidine strand for both sites.

This asymmetric intercalation binding geometry of the acridine moiety, stacked between two purine bases, and the concurrent polyamine location on the homopyrimidine phosphodiester backbone, provides a clear explanation of our DNase I protection data. Acridine and spermine show but small DNA sequence selective binding (see above), and neither molecule alone apparently exhibits the kind of sequence specificity shown by polyamine amide **1**. Based on computer simulation, this specificity seems to arise from acridine binding, and the polyamine plays the role of 'reporting' the specificity by binding to the homopyrimidine backbone and blocking DNase I cleavage. However, it is possible that the polyamine moiety plays a more direct role in determining the sequence selectivity of **1**, and we are currently synthesizing analogues of **1** to investigate this possibility further.

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Notes and References

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‡ Polyamine amide **1** was synthesized using N^1-mono-Boc-spermine[14] starting with spermine (free base) and Boc_2O (THF, 0–25 °C, 14 h, 54% after flash silica gel chromatography). Acylation with acridine-9-carboxylic acid [DCC (1.5 equiv.), cat. HOBt (0.05 equiv.), 48 h, DMF, 25 °C] afforded the desired mono-Boc protected conjugate as a viscous yellow oil (48% after flash silica gel chromatography). Deportection (TFA, 0 °C, 1 h) of the primary amine gave **1** as its poly-TFA salt, a yellow solid (100%) after lyophilisation.

§ The 197 base pair Hind III / Nhe I restriction fragment of pBR322 was labelled on the 3′-end of the bottom strand at the Hind III site (making base 34 the 3′-end of the bottom strand) with α-[^{32}P]dATP and reverse transcriptase (ref. 15). The labelled fragment was isolated, purified (PAGE, 6% non-denaturing gel), eluted from the gel with Tris–EDTA (TE) buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0), precipitated with EtOH and redissolved in TE buffer. DNA footprinting with polyamine amide **1** (0.2–25.0 μM) was performed using DNase I (0.01 units ml^{-1}) at 25 °C (4 min, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 20 mM NaCl, pH 7.5). The reaction was stopped using 80% formamide (5 μl) and cleavage products identified by co-electrophoresis with Maxam–Gilbert G-specific cleavage products on a denaturing gel (6% acrylamide, 8 M urea, 1500 V, 2 h).

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