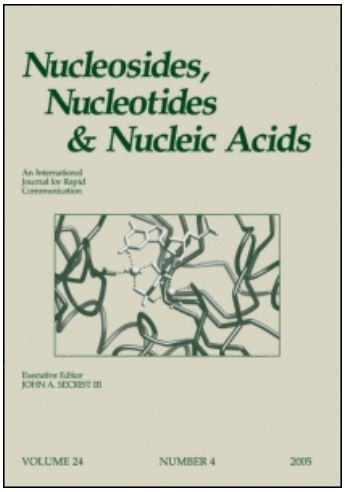

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Snyder, Ronald D.(1993) 'Effects of Polyamines on the Binding of Hoechst 33258 to Calf Thymus DNA', Nucleosides, Nucleotides and Nucleic Acids, 12: 1, 31 — 37
To link to this Article: DOI: 10.1080/07328319308016192
URL: http://dx.doi.org/10.1080/07328319308016192

NUCLEOSIDES & NUCLEOTIDES, 12(1), 31-37 (1993)

EFFECTS OF POLYAMINES ON THE BINDING OF HOECHST 33258 TO CALF THYMUS DNA

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ABSTRACT: The ability of polyamines to displace the minor groove-binding dye Hoechst 33258 from calf thymus DNA was investigated. Polyamines displace non-specific DNA phosphate bound Hoechst in a charge-dependent fashion, but show very little ability to displace the high affinity binding of Hoechst in the minor groove of DNA. This high affinity binding is, however, sensitive to ethidium bromide and the minor groove binding drug berenil. These studies suggest that polyamines probably bind DNA in the minor groove very weakly, if at all, relative to known minor groove binding agents.

The naturally occurring polyamines, putrescine, spermidine and spermine, have been shown to alter the physico-chemical properties of purified nucleic acids[1,2]. It has been difficult to measure effects of polyamines on DNA under physiological conditions in intact cells but the studies of Hung et al[3] have shown that the DNA of 9L rat brain tumor cells polyamine-depleted by treatment with α-difluoromethylornithine (DFMO) exhibited altered viscoelasticity and Snyder[4] demonstrated altered sensitivity of cellular DNA to DNAses in DFMO-treated HeLa cells. These latter studies suggest that polyamines probably do play a role in chromatin structure and that a better understanding of their interactions with DNA might prove useful not only in elucidating structural and regulatory roles of polyamines but possibly also in the design of synthetic polyamines with antiproliferative activity.

Several models for polyamine:DNA binding have been proposed based either on counterion condensation theory[5] which by itself can explain polyamine induced DNA condensation and aggregation phenomena, or site-

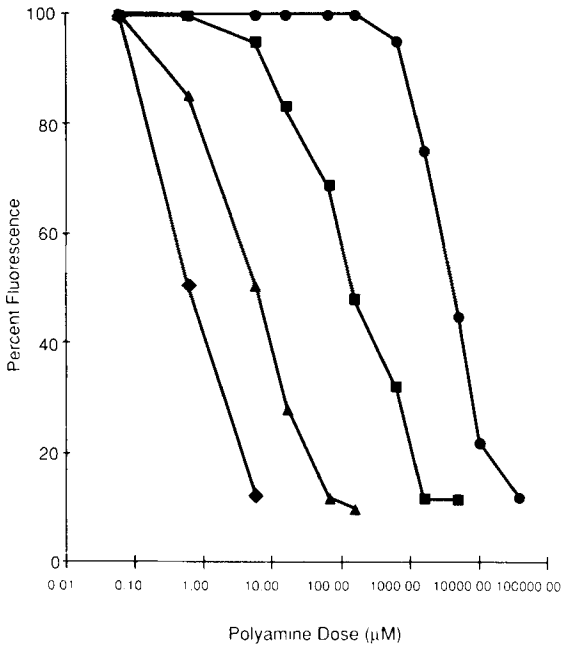

FIG. 1. Displacement of ethidium bromide from calf thymus DNA by polyamines. Flourescence reduction of ethidium/DNA complex was measured using 1.6 μM ethidium bromide and 2.0 μM DNA phosphate at emission and excitation wavelengths of 598 and 546 nm, respectively as described in detail previously[10]. Pentaamine 4-4-4-4 was obtained from H. Basu. ● Putrescine; ■ Spermidine; ▲ Spermine; ◆ Pentaamine.

specific interaction[6-8]. Based on data derived from X-ray crystallographic analyses and energy minimization considerations, Feuerstein et al theorized that the most energetically favorable interaction of spermine with DNA would be in the major groove at alternating purine/pyrimidine sequences[9]. Phosphate backbone and minor groove binding appeared to be less favored although the energy distinctions were concluded to be dependent on the sequence[9]. In an attempt to learn more about these interactions we investigated whether polyamines could displace Hoechst 33258 dye from calf thymus DNA under conditions in which dye bound predominantly to the DNA phosphate backbone or in the minor groove.

We have previously observed that polyamines can displace the intercalating dye ethidium bromide from DNA and that this displacement

TABLE 1. Emission and excitation maxima for Hoechst 33258 binding to calf thymus DNA under various conditions.

Conditions	λ_{max} excitation	λ_{max} emission
0.05 dye/DNA no salt	360	477
+ 33 μM spermine	350	454
0.005 dye/DNA no salt	360	470
0.0005 dye/DNA no salt	360	470
+ 330 μM spermine	350	458
0.05 dye/DNA 100 mM NaCl	350	457
+ 33 μM spermine	350	457
0.005 dye/DNA 100 mM NaCl	350	459
+ 330 μM spermine	350	461

Emission and excitation maxima were determined on an SLM Aminco SPF-500C spectrofluorometer equipped with spectral analysis software. Spermine concentrations chosen were those which maximally reduced fluorescence of the dye/DNA complex. Dye/DNA ratio on a molar basis.

can be driven to near completion[10]. FIG. 1 shows that the net charge of the polyamine determines the efficacy of this displacement with approximate IC_{50}s being 500 μM, 90 μM, 6 μM and 0.8 μM for the diamine, triamine, tetramine and pentaamine, respectively. A similarly ordered charge dependency was observed by Stewart[11]. This ability of non-intercalative drugs to displace intercalating agents from DNA has been well investigated[12] and has been found on theoretical grounds to most likely relate to minor groove binding. Agents which might be presumed to bind DNA only at the phosphate backbone, however, were excluded from this analysis. Thus it is not clear that groove binding is a prerequisite for ethidium bromide displacement.

Hoechst 33258 forms at least five complexes with calf thymus DNA with binding affinities defined by conditions of ionic strength and molar dye/DNA ratios and with accompanying spectral shifts[13,14]. TABLE 1

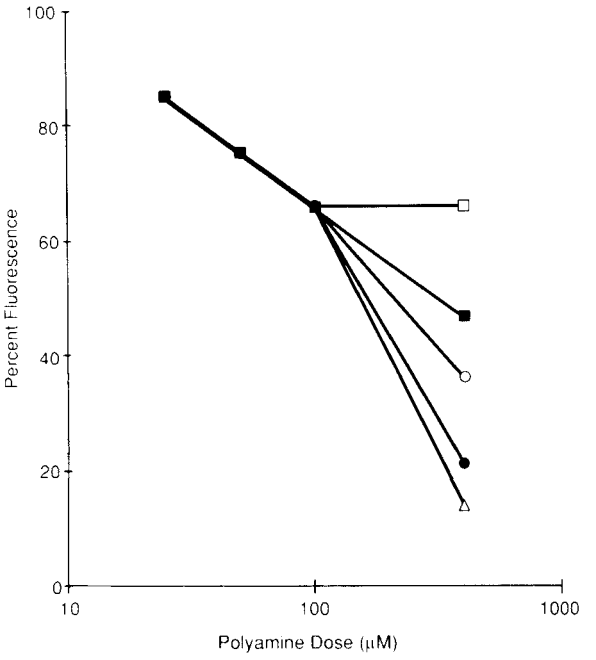

FIG. 2. Displacement of Hoechst 33258 from calf thymus DNA at low ionic strength. Fluorescence reduction of Hoechst/DNA complex was measured using 20-200 nmoles DNA phosphate and 0.3 to 3.0 nmoles Hoechst 33258 in 1 mM NaCl, 1 mM Tris-EDTA (7.3) at emission and excitation wavelengths of 365 and 470 nm, respectively. Spermidine was either added alone up to 600 μM (□) or titrated up to 100 μM with subsequent additions of 0.42 μM (■), 0.82 μM (○) or 2.9 μM (△) ethidium bromide or 4.4 μM Berenil (●).

demonstrates the shifts of both emission and excitation maxima as ionic strength and dye/DNA ratio are varied. High ionic strength and low dye/DNA ratio reduce non-specific DNA phosphate binding, leaving only high affinity binding in the minor groove.

As shown in FIG. 2, under low salt conditions (1 mM NaCl) spermidine can maximally displace only about 35% of Hoechst dye as judged by decrease in DNA:dye fluorescence regardless of concentration. By contrast, ethidium bromide and the presumed minor-groove binding drug berenil[15] nearly completely displace the Hoechst. None of the polyamines tested was able to remove more than about 35% of the Hoechst dye bound either at low salt or high dye/DNA ratios as shown in FIG. 3A.

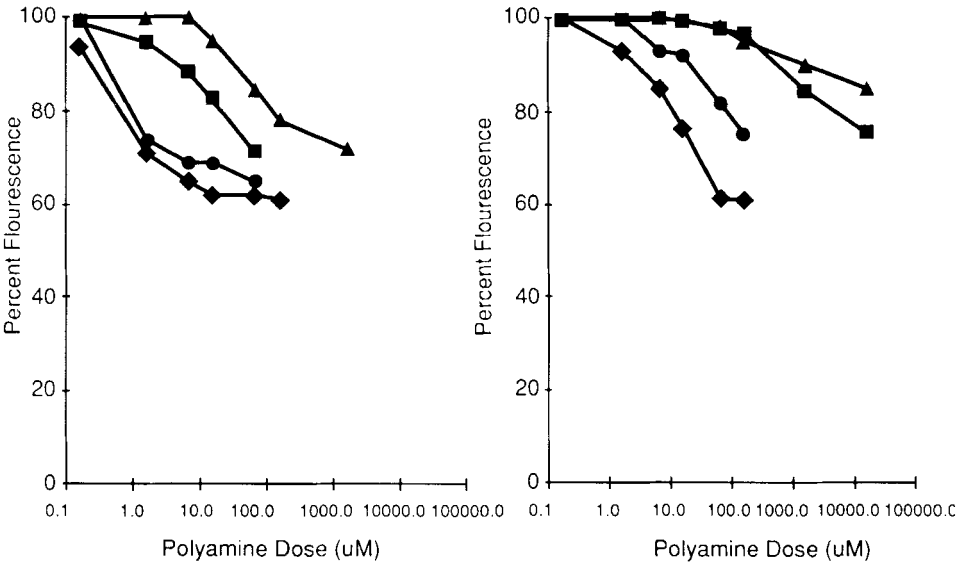

FIG. 3. Titration of Hoechst/DNA binding by polyamines in the presence of 1 mM (A) or 100 mM NaCl (B). Spectral parameters as in legend to FIG. 2. ▲ Putrescine, ■ Spermidine, ● Spermine, ◆ Pentaamine.

Consistent with the ethidium displacement studies (FIG. 1), however, there was an increasing ability to displace Hoechst as total polyamine charge increased. Under high salt conditions (100 mM NaCl) shown in FIG. 3B, one to two log higher polyamine concentration was required for dye displacement and the maximum decrease in fluorescence was generally less than under low salt conditions. The existence of at least two high affinity binding sites is suggested by the observation that even under high salt conditions in which there is no evidence for non-specific Hoechst binding as judged by spectral characteristics, all of the polyamines can still remove a fraction of dye (FIG. 3B) albeit with much lower potency.

FIG. 4 demonstrates that Hoechst displacement by polyamines is also dependent on dye/DNA ratio. At low ratios of 0.005 (and 1 mM NaCl), 3.3 μM spermine reduces the fluorescence of the dye/DNA complex by only about 3% whereas at a dye/DNA ratio of 0.05 maximal displacement of about 35% is observed. As shown in TABLE 1, maximal displacement of

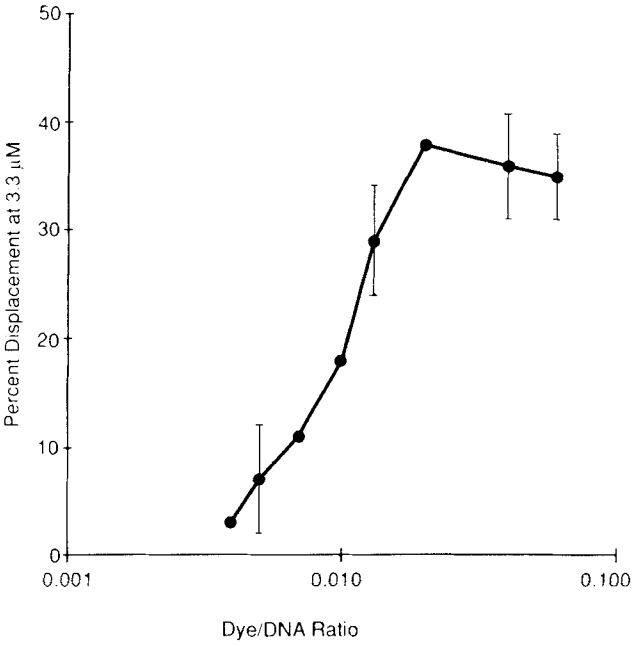

FIG. 4. Dependence of dye/DNA ratio on the ability of spermine to reduce the fluorescence of Hoechst/DNA complex. Spermine was added to 3.3 μM final concentration to dye/DNA complexes of different molar ratios and fluorescence reduction was measured as described in the legend to FIG.2.

Hoechst by polyamines under conditions of low salt or high dye/DNA ratio results in a complex with spectral characteristics similar to that found under high salt or low dye/DNA ratio. Addition of polyamines to dye/DNA complex at high ionic strength or low dye/DNA ratio produced no further spectral shifts.

These data are consistent with the interpretation that polyamines can readily displace non-specifically bound (phosphate backbone) Hoechst 33258 but that they are basically incapable of removing the majority of Hoechst bound in the minor groove of DNA. Since this high affinity binding is readily displaced by both intercalating agents and the minor groove binding drug berenil, it must be concluded that naturally occurring polyamines probably interact only sparingly with the minor groove of DNA relative to, at least, berenil.

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Received 5/12/92
Accepted 9/24/92