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Ability of spermine to differentiate between DNA sequences—Preferential stabilization of A-tracts

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Abstract

The regulatory roles fulfilled by polyamines by governance of chromatin structure are made possible by their strong association with cellular DNA, and hence by their ability to modulate DNA structure and function. Towards this end, it is crucial to understand the manifestation of sequence-dependent polyamine binding at the secondary and tertiary structural levels of DNA. This study utilizes circular dichroism (CD) and isothermal titration calorimetry (ITC) to address this relationship by using 20 bp oligonucleotides with sequences–poly(dA):poly(dT), poly (dAdT):poly(dAdT), poly(dG):poly(dC), poly(dGdC):poly(dGdC)–that yield physiologically relevant structures, and poly(dIdC):poly(dIdC). CD studies show that at physiological ionic strength (150 mM NaCl), spermine preferentially stabilizes A-tracts, and increases flexibility of the G-tract oligomer; the latter is also suggested by the larger change in entropy (ΔS) of spermine binding to G-tracts. Given the chromatin destabilizing property of these sequences, these findings suggest a role for spermine in stabilization of non-nucleosomal A-tracts, and a compensating mechanism for incorporation of G-tracts in the chromatin structure. Other implications of these findings in sequence dependent DNA packaging are discussed.

Keywords: Sequence-specific spermine–DNA binding; Isothermal Titration Calorimetry (ITC); A-tracts; G-tracts; Thermodynamics of spermine–DNA binding

1. Introduction

The omnipresence of polyamines in mammalian cells has implications in functions ranging from gene expression to cell growth regulation [1–4]. These functions are made possible by intracellular concentrations of polyamines, such as spermine and spermidine, in the millimolar range—owing to their charged state at physiological pH they are substantially bound to nucleic acids, thereby modulating the biological activity of DNA [5,6]. Spermine, a tetracationic polyamine, binding to DNA manifests itself at the secondary and tertiary structural levels, thereby influencing transcriptional functionalities by affecting the ability of other ligands, e.g., transcription and regulatory enzymes, to compete for binding sites on DNA strands. This can be affected by induction of a B → Z transition in polynucleotides by spermine [7–9]. The presence and concentration of spermine in the chromatin can stabilize [10], or destabilize double stranded DNA, and thereby influence chromosomal DNA packaging [4,11–13]. Using this polyamine as a model, we strive to understand the relationship between DNA secondary structure, as influenced by nucleotide sequence, and thermodynamics of spermine binding to duplex DNA. A deeper understanding of such interactions would also aid in establishing the role of nucleotide sequence in multivalent cation-induced DNA packaging for gene delivery applications.

It is imperative to study spermine–DNA interactions as a function of nucleotide sequence because of its role in chromatin structure and in other biological functions: oligopurines tracts of 10 or more contiguous residues are found 4 times more frequently in the β-globin region of human genomic DNA than would be expected with a random distribution of sequences [14]; 10 to 30 bp long A-tracts occur frequently in eukaryotic

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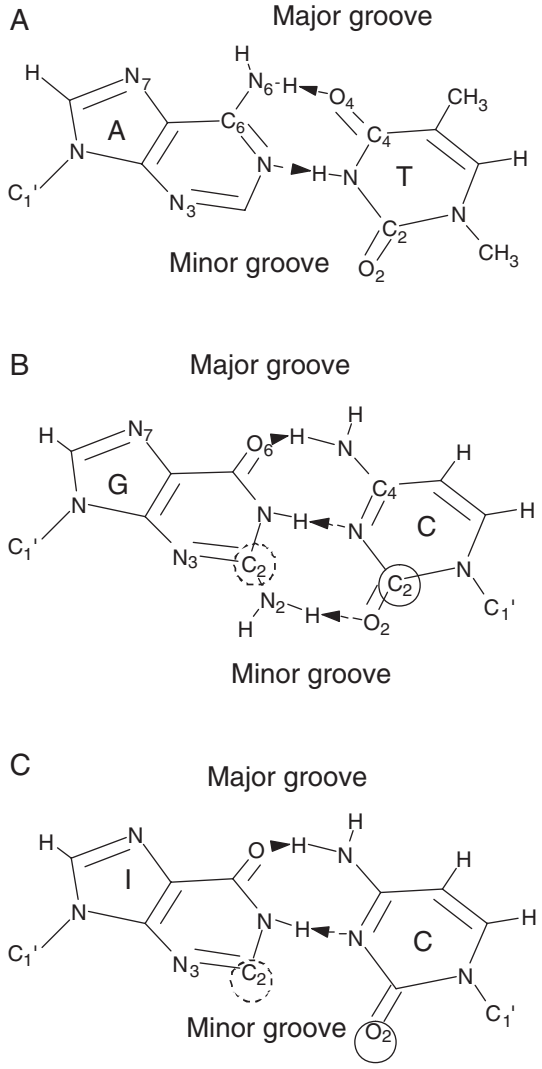

Fig. 1. Structures of (A) A–T, (B) G–C, and (C) I–C base pairs—the missing amine group at C_2 position (dashed circle) can be used to test a potential steric hindrance to spermine binding to the minor groove of G–C base pairs, i.e., to N3 of guanine and O2 of cytosine (full circle).

genomes [15]; the alternate AT sequence (AT/TA motif) is of broad biological significance, illustrated by its function in the regulation of gene transcription in both eukaryotes (TATA box) and prokaryotes (TATAAT, −10 consensus sequence) [16]; GC sequences are known to be hot spots for mutations in DNA—a G→T mutation in a run of seven G's near the 5′ splice site of intron 3 gives rise to the Wiedemann–Beckwith syndrome [17]; telomeres, the end caps of chromosomes, have simple repetitive sequences with one strand G-rich (12–16 bases) relative to the other, C-rich, strand [18,19]. Thus, a systematic study of spermine-induced differential stability of DNA duplexes and associated binding thermodynamics, as a function of nucleotide sequence, may provide insight into the role of spermine in a variety of cellular processes.

Most reports on sequence-dependence of spermine binding to DNA have used small oligomers (hexamers or octamers) [20–23], where base-induced local helix bending can complicate interpretations regarding global secondary structural changes, e.g., B→A transitions [24]. However, recent work probing the structure of DNA within cationic lipid–DNA complexes has utilized 20 bp long oligomers [25]. We have used 20 bp oligomers comprised of (purine:pyrimidine)$_n$ homooligomers and alternate purinepyrimidine oligomers that yield biologically relevant secondary and tertiary structures. They offer the advantage of having two helical turns (~10.2 bp/turn), and hence their overall structure is less dependent on artifacts introduced by base-induced local bending as in shorter oligomers. Thus, sequence-dependent structural changes in DNA as a result of spermine binding, and the corresponding thermodynamic driving forces can be isolated in physiologically relevant structures.

Spermine has been reported to have weak or no base-pair selectivity [26,27] or GC selectivity [28–31] in DNA duplexes. A minor-groove binding site of spermine in AT-rich DNA has been suggested from photoaffinity cleavage studies [32,33]. In mixed sequences spermine binding is diffusive, wherein it shifts between the minor groove of B-DNA (AT rich DNA) and the major groove of A-DNA (G-tracts) [32]. Theoretical studies predict binding of spermine to the major groove of GC-rich duplexes [30,34], and to the minor groove of AT-rich duplexes [35,36]. Potential binding sites for spermine in GC rich DNA are N7 and O6 of guanine in the major groove [37]. We have tested the hypothesis that spermine could bind to N3 of guanine and O2 of cytosine in the minor groove if not for the presence of the guanine N2 amino group (Fig. 1B). According to this hypothesis, inosine–cytosine duplexes should allow binding of spermine to its minor groove, since the IC and GC base pairs differ only by the guanine N2 amino group (Fig. 1C). We test this hypothesis by comparing the energetics of spermine binding to these oligomers by ITC, and monitoring corresponding structural changes by CD. The ability of spermine to induce differential stabilization in polynucleotides has also been studied by thermal melting of the oligonucleotides as a function of spermine concentration. In addition, we address the ability of spermine to discriminate between homooligomers and alternating oligomers by studying secondary and tertiary structure dependent energetics of spermine binding to these sequences.

2. Materials and methods

Single stranded oligonucleotides (20 b)–poly(dA), poly(dT), poly(dG), poly(dC), poly(dAdT), poly(dGdC), and poly(dIdC) – were purchased from Invitrogen Corp. (Carlsbad, CA), in cartridge purified form. The purification process is based on reverse phase chromatography and serves to remove failed sequences after complete synthesis. HEPES (4-(2-Hydroxyethyl)-1-piperazineethane-sulfonic acid), EDTA (ethylenediamine tetra-acetic acid), and spermine tetrachloride were purchased from Sigma Chemical Co. (St. Louis, MO) in powder form and used without further purification.

2.1. Oligonucleotide annealing

Oligonucleotides were solubilized in 5 mM HEPES, 1 mM EDTA, and 150 mM NaCl buffer (pH 6) filtered through a